Prenatal Exposure, Maternal Sensitization, and Sensitization *In Utero* To Indoor Allergens in an Inner-City Cohort

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Primary sensitization to antigens may occur prenatally. We hypothesized that high prenatal exposure to indoor antigens increases the risk for sensitization in newborns in New York City populations with increased risk for asthma. We also investigated whether maternal sensitization is required for *in utero* sensitization to occur. One hundred sixty-seven pregnant African American or Dominican women residing in northern Manhattan were recruited and antigen was measured from home dust. After delivery, newborn cord and maternal blood were assayed for IgE and mononuclear cell proliferation and cytokine production in response to antigen. Cockroach, mouse, but not dust mite antigens, were commonly elevated in the kitchens and pregnant mothers' beds. Increased mononuclear cell proliferation occurred in 54% of newborns in response to cockroach, 25% in response to dust mite *Dermatophagoides pteronyssinus*, 40% in response to dust mite *D. farinae*, and 34% in response to mouse protein extracts. Antigen-induced mononuclear cell proliferation occurred in cord blood even in the absence of antigen-induced mononuclear cell proliferation in the mother. Proliferation in response to antigens did not correlate with IgE levels, but proliferation in response to dust mite extracts correlated with interluekin-5 (IL-5) production in cord blood. These results suggest that (*1*) high prenatal exposures to cockroach and mouse antigens are prevalent; (*2*) *in utero* sensitization to multiple indoor antigens is common, occurs to a different degree than maternal sensitization, and may involve IL-5 upregulation.

Keywords: *in utero* sensitization; indoor antigens; lymphocyte proliferation

Asthma morbidity and mortality have increased significantly in the United States since the late 1970s (1, 2). The most substantial increases have occurred among children zero to 4 yr of age (1) and among urban ethnic minorities (1, 3). These ethnic disparities are prominent in New York City (NYC), where hospitalization and death rates from asthma are as much as five times greater among non-Latino African Americans and Latinos than among non-Latino white individuals (2).

Exposure to dust mite allergen has been shown to increase the risk for the onset of asthma (4), and reductions of dust mite allergen have been shown to reduce asthma morbidity (5). Although exposure to several other indoor allergens or antigens (i.e., derived from cats, dogs, and fungi) has been hypothesized to contribute to the development of asthma, exposure and sensitization to cockroach antigens has emerged as playing a significant role in several U.S. inner cities (6, 7). High levels of cockroach antigen in apartments housing children 3 mo of age born to asthmatic/allergic parents increased the risk of repeated wheezing episodes by 1 yr of age (8), and increased T-cell reactivity in response to cockroach antigen at 2 yr of age (9). Moreover, children with elevated total serum immunoglobulin E (IgE) levels at 9 mo of age were predisposed to persistent wheezing and positive allergy skin tests by 6 yr of age (10), suggesting that critical exposures to antigens may occur at a very young age.

Prenatal antigen exposure may lead to sensitization in the newborn. Very low levels of total IgE have been measured at birth, yet their presence does not predict persistent wheezing during childhood (11). Instead, studies have utilized cord blood mononuclear cell (CBMC) proliferation assays for T-cell reactivity in response to specific antigens as a surrogate measure for sensitization. In adults, such proliferation assays reflected T-cell responses (12), and in the case of dust mite proteins, correlated with skin test sensitivity (13). Thus far cord blood mononuclear cells have been shown to proliferate after *in vitro* stimulation to tree, grass, birch, cat, and dust mite antigens (14, 15). Dust mite and ovalbumin-specific T-cell clones have been derived from cord blood. Genotyping has demonstrated that these antigen-specific clones are fetal in origin (16).

These results suggest that sensitization and the development of T-cell memory may occur prenatally. The prevalence of in *utero* sensitization to indoor antigens, predisposing factors, and mechanism and eventual clinical relevance is not known. We hypothesized that high exposure to indoor antigens during the prenatal period in NYC populations with increased asthma morbidity rates is important to antigen sensitization in newborns. We also investigated whether CBMC proliferation in response to antigens occurs even in the absence of maternal sensitization.

METHODS

Patients

The cohort consisted of 167 pregnant women, self-reported as African American or Dominican and residing in Harlem, Washington Heights, or the South Bronx of NYC. Over 19 mo pregnant women were recruited from clinics affiliated with Columbia University as part of an ongoing study under the auspices of the Columbia Center for Children's Environmental Health. Exclusion criteria for women included smoking, illicit drug use, diabetes, hypertension, HIV infec-

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tion, younger than 18 or older than 35 yr of age, and residence in New York City for less than 1 yr prior to pregnancy. The study was approved by the Institutional Review Board, and written informed consent was obtained from all participants.

Dust Collection, Antigen Measurement, and Blood Collection

Dust samples from a subset of 74 homes during the first 12 mo of recruitment were analyzed for inhalant antigens in the approximate order they were enrolled. Most samples were collected prenatally (n = 68; mean, 43 d; range, 3 to 82 d prepartum), and six were collected postpartum (mean, 26 d; range, 4 to 82 d). Dust samples were vacuumed separately from the kitchen and pregnant mother's bed (17). Mouse urinary protein was assayed with a competitive enzyme-linked immunosorbent assay (ELISA) using a mouse urine mixture (containing *Mus m* 1), a polyclonal antibody (rabbit antimouse) (Greer Laboratories, Inc., Lenoir, NC), and a polyclonal goat antirabbit antibody (Sigma Chemical Co., St Louis, MO). Dust mite (*Der f* 1 and *Der p* 1) and cockroach antigens (*Bla g* 2) were assayed by ELISA (Indoor Biotechnologies, Clwyd, UK) (18).

Cord blood was collected at delivery and maternal blood within 1 d postpartum. Fresh mononuclear blood cells were separated by density centrifugation (19) and plated in triplicate for proliferation assays and in duplicate for cytokine assays. In 12 cases, insufficient blood was obtained for mononuclear cell isolation.

Proliferation, IgE, and Cytokine Assays

Mononuclear cells were cultured in microtiter plates as described (13, 15). Cells were cultured for 5 d with phytohemagglutinin (PHA) positive control (10 μg/ml) (Sigma), German cockroach (10 μg/ml), *Dermatophagoides pteronyssinus* (10 μg/ml), *D. farinae* (10 μg/ml), mouse protein extract (10 μg/ml) (Greer Laboratories), and tetanus toxoid (1:500 dilution) (Wyeth-Lederle Vaccines and Pediatrics, Pearl River, NY) or no antigen. T-cell reactivity in response to *D. pteronyssinus* protein extracts (Greer Laboratories) with that in response to monoclonal affinity-purified *Der p* 1 and *Der p* 2 proteins (10 μg/ml) (kindly furnished by Martin Chapman, University of Virginia) in subset of cord (n = 51) and maternal (n = 57) specimens also was compared. Proliferation was measured by thymidine incorporation (20). Increased proliferation in response to antigen was defined as (*1*) Stimulation Index (SI) (averaged counts per minute [cpm] in the presence of antigen divided by averaged cpm without antigen) greater than 2, and (*2*) antigen-induced averaged cpm greater than 1,000 above background (14, 15). A separate aliquot of 3×10^5 cells/well was cultured simultaneously with PHA, cockroach, dust mite, mouse extract, or no antigen under identical conditions, and supernatants were collected on Day 5 for cytokine analysis.

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION*

Characteristics	%
Mother's ethnicity	
Dominican	56.9% (95/167)
African American	39.5% (66/167)
Other	3.6% (6/167)
Father's ethnicity	
Dominican	46.7% (78/167)
African American	29.9% (50/167)
Other	23.4% (39/167)
Mothers with history of:	
Asthma	22.2% (29/132)
Allergic rhinitis	6.9% (9/131)
Atopic dermatitis	8.4% (11/131)
Fathers with history of:	
Asthma	10.8% (14/130)
Allergic rhinitis	3.1% (4/129)
Atopic dermatitis	5.6% (7/125)
Mothers who:	
Live in apartments	95.2% (159/167)
Spend > 7 h/day awake indoors	73.0% (119/167)
Received tetanus immunization within last 10 yr	78.9% (60/76)

* Data obtained by questionnaire given to the pregnant mothers. Denominators indicate the numbers of patients for whom data were available.

Total serum levels were measured by immunoradiometric assay (Total IgE IRMA; Diagnostics Products Corp., Los Angeles, CA). Allergen-specific IgE levels were measured by the Fluorescence Allergosorbent Test (FAST) (BioWhittaker, Walkersville, MD) (21). Interleukin-5 (IL-5) and interferon gamma (IFN-γ) levels from culture supernatants were measured by ELISA (Immunotech, Marseille, France). All specimens were analyzed in duplicate.

Statistical Analysis

Nonparametric statistical analyses were performed as described (22), except where noted. Levene's test for equality of variance was utilized to determine the appropriate form of *t* test prior to parametric testing. Exposure/biomarker relationships were assessed by categorizing the dustborne antigen into four groups (below detectable limits, 0 to 2, 2 to 8, > 8 μg or Ug/g) and performing ANOVA for trends (SPSS statistical software, Chicago, IL). Comparisons between proliferation in response to *D. pteronyssinus* protein extract (containing *Der p* 1 and *Der p* 2 proteins) and purified *Der p* 1 or *Der p* 2 protein were made as described (*see* APPENDIX).

RESULTS

Characteristics of the study population are shown in Table 1. Cockroach and mouse antigen was commonly recovered from kitchen samples and, to a lesser extent, the pregnant mother's bed (Figure 1, *top* and *middle panels*). *Der f* 1 and *Der p* 1

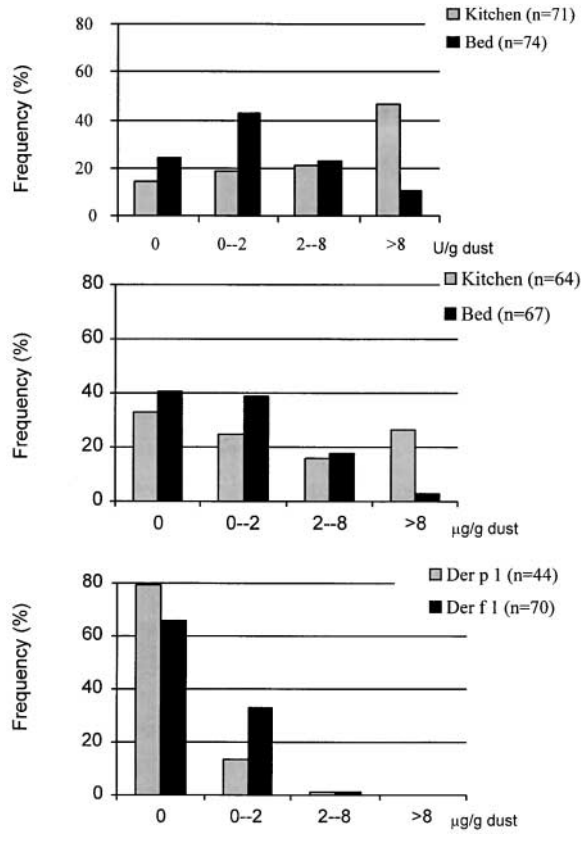

Figure 1. Frequency of antigen in settled dust in both the kitchen and the pregnant mother's bed. Y axis refers to percent of affected homes. X axis refers to allergen measurements in settled dust. Zero refers to any value below the limit of detection of the assay. (*Top panel*) German cockroach antigen, as represented by Bla g 2 levels in kitchen and pregnant mother's bed. The limit of detection of the assay was 0.025 U/g dust. (*Middle panel*) Mouse urinary antigen in kitchen and pregnant mother's bed. The limit of detection of the assay was 0.082 μg/g dust. (*Bottom panel*) Dust mite antigens *Der p* 1 and *Der f* 1 in pregnant mother's bed. The limit of detection of the assay was 0.025 μg/g dust.

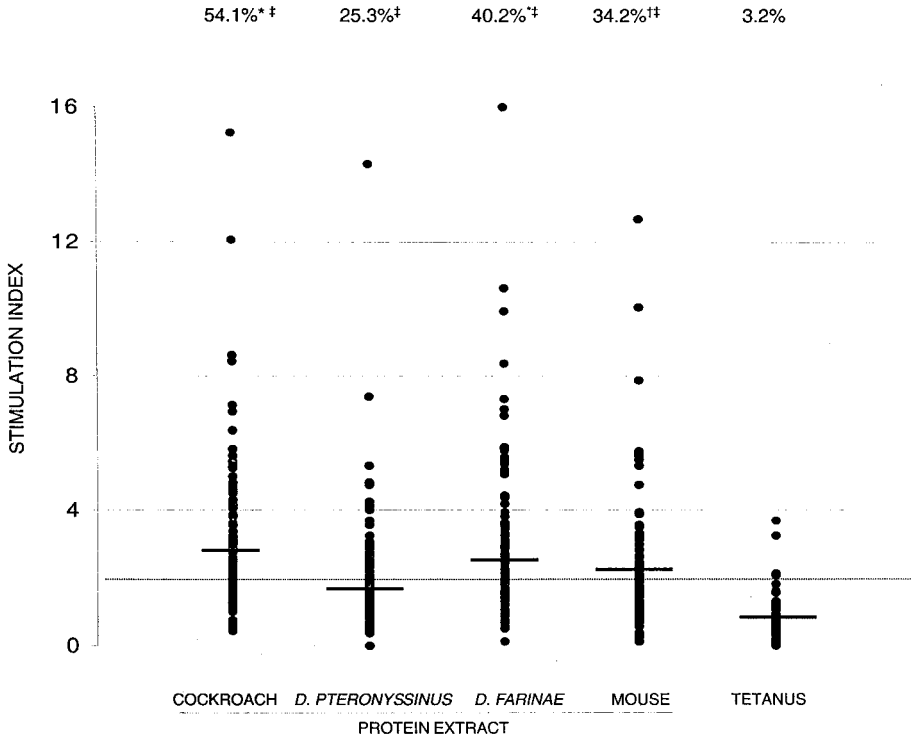

Figure 2. Cord blood proliferation in response to several antigens. Stimulation Index (SI) refers to averaged counts per minute (CPM) in response to antigen divided by averaged CPM in the absence of antigen. Reported rates refer to the percentage of samples with both a SI $>$ 2 and averaged counts per minute 1,000 above background. *Bars* represent mean values. *p value $<$ 0.0005 compared with *D. pteronyssinus* extract, †p value $<$ 0.05 compared with *D. pteronyssinus* extract, ‡p value $<$ 0.0005 compared with tetanus (McNemar Test).

were below 2 μg/g dust in 99 and 93% of the beds, respectively (Figure 1, *bottom panel*).

Mononuclear Cell Proliferation and Cytokine Production in Response to Indoor Antigens

The SI was increased in 54% of newborns in response to cockroach extract, 25% in response to *D. pteronyssinus* extract, 40% in response to *D. farinae* extract, 34% in response to mouse extract, and 3% in response to tetanus toxoid (Figure 2). We observed a high degree of correlation between the T-cell proliferation in response to the two dust mite species (Spearman's rho, r = 0.581; p $<$ 0.001), possibly in part reflecting their extensive cross-reactivity (23). Because sensitization to tetanus only occurs in offspring of mothers immunized during pregnancy (24), our finding that cord blood proliferation was significantly more common to all tested antigens than to tetanus (p $<$ 0.0005, McNemar Test) supports the likelihood that nonspecific T-cell reactivity and significant maternal blood contamination are not present. In addition, cord blood proliferation in response to cockroach, *D. farinae* (p $<$ 0.0005), and

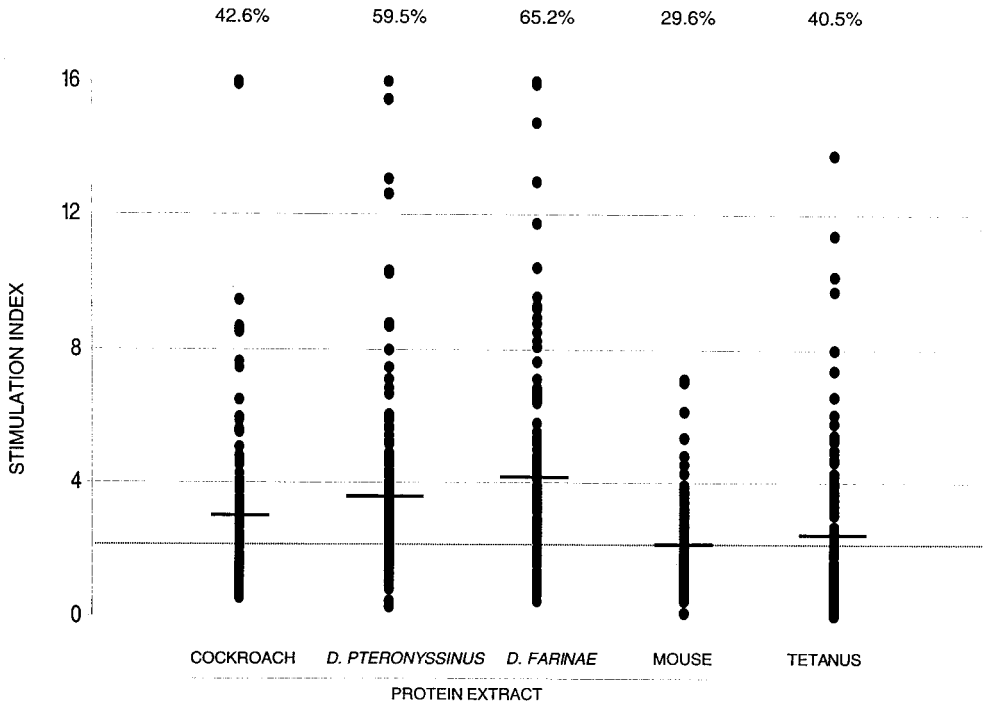

Figure 3. Maternal blood proliferation in response to several antigens. Stimulation Index (SI) refers to averaged CPM in response to antigen divided by averaged CPM in the absence of antigen. Reported rates refer to the percentage of samples with both the SI $>$ 2 and averaged counts per minute 1,000 above background. *Bars* represent mean values.

TABLE 2A. COMPARISON OF MEDIAN IL-5 LEVELS (PG/ML) PRODUCED IN RESPONSE TO ANTIGEN WITH LEVEL PRODUCED UNDER NONSTIMULATED CONDITIONS

	(*n*)	Median	25th Percentile	75th Percentile	p Value
PHA	62	75.60	22.45	164.85	< 0.0005
Cockroach Extract	62	46.59	7.95	118.33	< 0.0005
D. pteronyssinus Extract	58	5.34	1.07	37.21	< 0.0001
D. farinae Extract	61	12.82	1.56	66.05	< 0.0005
Mouse Extract	59	18.34	1.54	50.49	< 0.0005

TABLE 2B. COMPARISON OF MEDIAN IFN-γ (PG/ML) PRODUCED IN RESPONSE TO ANTIGEN WITH LEVEL PRODUCED UNDER NONSTIMULATED CONDITIONS

	(*n*)	Median	25th Percentile	75th Percentile	p Value
PHA	72	209.45	40.56	938.75	< 0.0005
Cockroach Extract	72	296.90	34.78	1111.66	< 0.0005
D. pteronyssinus Extract	64	18.75	4.76	62.01	NS
D. farinae Extract	72	43.19	7.00	214.65	< 0.005
Mouse Extract	68	23.77	6.39	82.55	NS

Comparison of IL-5 (Table 2A) and IFN-γ (Table 2B) levels produced in response to antigen with levels produced under nonstimulated conditions. Analysis performed using Sign test for two related variables; p values provided are for two-tailed significance. For IL-5, levels are compared with median (50th percentile) background level 2.11 (25th percentile 0; 75th percentile 9.04 pg/ml). For IFN-γ, levels are compared with median background level 20.70 (25th percentile 6.14; 75th percentile 55.48 pgml).

mouse ($p < 0.05$) extract was significantly more frequent than in response to *D. pteronyssinus* extract, the protein associated with the lowest level of detectable exposure in the homes. As a control for possible nonspecific mitogenic effects of the protein extracts (12, 13), we found significant correlations between T-cell reactivity in response to *D. pteronyssinus* protein extract with that observed in response to monoclonal purified *Der p*1 (n = 49, Spearman's rho, r = 0.659; p value < 0.0005) and *Der p* 2 protein (n = 39, Spearman's rho, r = 0.432; p value < 0.01). This result implies that the neonatal T-cell reactivity occurred in specific response to dust mite antigen.

Increased maternal blood mononuclear cell proliferation was seen in 43% in response to cockroach extract, 60% in response to *D. pteronyssinus* extract, 65% in response to *D. farinae* extract, 30% in response to mouse extract, and 41% in response to tetanus toxoid (Figure 3). Similar to our observations in newborns, T-cell reactivity in response to indoor antigens was common, and strong correlations were found between the *D. pteronyssinus* and *D. farinae* extract-induced responses (Spearman's rho, r = 0.798; p < 0.0005). In addition, proliferation in response to *D. pteronyssinus* protein extract correlated significantly with that obtained in response to monoclonal purified *Der p*1 (n = 56, Spearman's rho, r = 0.509; p value < 0.0005) and *Der p* 2 protein (n = 48, Spearman's rho, r = 0.334; p value < 0.05). The mean SI in response to *D. pteronyssinus* extract, *D. farinae* extract, and tetanus was significantly greater ($p < 0.0005$, *t* test for comparison of means) than that observed in newborns. Finally, a reported history of tetanus immunization within the previous 10 yr was associated with tetanus-induced T-cell reactivity in the mothers (Fisher's exact test, two-tailed $p < 0.05$).

We found no significant correlation between either cord- or maternal-total IgE and proliferation in response to any individual or group of antigens (data not shown). Although allergen-specific IgE was not measurable in cord blood, cockroach and dust mite-specific IgE levels in maternal blood were only weakly associated with proliferation in response to the same antigen (r = 0.194, p = 0.035; r = 0.177, p = 0.056; respectively). These results suggest that while T-cell reactivity in response to indoor antigens may represent a biomarker for exposure, it may not necessarily signify an IgE-mediated allergic response.

To distinguish whether T-cell proliferation in response to antigens reflects an allergic response or a T-cell-mediated immune response to a previous exposure, we compared newborn T helper 2 (i.e., IL-5) cytokine production in response to antigen stimulation with T helper 1 (i.e., IFN-γ) levels. The median IL-5 level after cord blood stimulation with PHA, cockroach extract, dust mite extract, and mouse extract was significantly greater than that observed in the absence of stimulation with antigen. Similar increases in the median IFN-γ levels were observed in response to PHA, cockroach extract, and *D. farinae* extract (Tables 2A and 2B). Increased IL-5 (Tables 3A and 3C) and less consistently IFN-γ (Tables 3B and 3D) production in specific association with increased T-cell proliferation was observed only after stimulation with dust mite extract, suggesting that allergic cytokine responses occur only in restricted cases.

Relationship between Antigen-induced Proliferation and Indoor Antigen Levels

Seasonally dependent cord blood reactivity in response to outdoor allergens has been described (25), suggesting that a relationship between prenatal environmental exposure and sensitization may exist. We found no significant association, however, between cockroach antigen levels in the kitchen or pregnant mother's bed and cockroach extract-induced proliferation and cockroach-specific IgE in either maternal or cord blood (data not shown). Similarly, such associations were absent for dust mite antigen in settled dust and maternal and cord blood biomarkers. In contrast, increased maternal, but not cord blood, lymphocyte proliferative responses to mouse extract was associated with higher levels of mouse antigen in kitchen dust (for trend, $p < 0.05$). In particular, mouse antigen levels greater than 3 μg/g dust in the kitchen were associated with increased maternal T-cell reactivity to mouse extract

TABLE 3A. COMPARISON BETWEEN SI POSITIVITY AND MEDIAN IL-5 LEVEL IN RESPONSE TO ANTIGEN*

	SI	Median IL-5 Level	25th Percentile	75th Percentile	Mann-Whitney U	p Value
PHA (n = 62)	Negative	93.42	9.71	162.74	229.00	NS
	Positive	72.26	24.72	165.39		
Cockroach Extract (n = 62)	Negative	49.59	6.85	138.96	1380.50	NS
	Positive	39.84	7.70	118.58		
D. pteronyssinus Extract (n = 38)	Negative	1.51	0	13.71	967.00	< 0.005
	Positive	27.44	3.87	89.95		
D. farinae Extract (n = 61)	Negative	5.14	0.60	15.95	268.00	< 0.005
	Positive	22.51	8.41	129.22		
Mouse Extract (n = 59)	Negative	8.83	0.39	59.29	406.50	NS
	Positive	18.95	2.10	46.58		

* Analysis performed using Mann-Whitney test. The SI is considered positive if greater than or equal to 2.

TABLE 3B. COMPARISON BETWEEN SI POSITIVITY AND MEDIAN IFN-γ LEVEL IN RESPONSE TO ANTIGEN

	SI	Median IFN-γ Level	25th Percentile	75th Percentile	Mann-Whitney U	p Value
PHA (n = 72)	Negative	47.40	13.40	356.90	262.00	NS
	Positive	246.95	46.91	960.50		
Cockroach Extract (n = 72)	Negative	217.78	32.16	982.66	503.50	NS
	Positive	334.40	44.09	1268.75		
D. pteronyssinus Extract (n = 64)	Negative	16.92	5.19	58.64	316.00	NS
	Positive	34.30	12.59	91.60		
D. farinae Extract (n = 72)	Negative	26.28	3.52	91.73	353.50	< 0.005
	Positive	99.05	28.56	446.14		
Mouse Extract (n = 68)	Negative	19.00	5.75	75.38	465.50	NS
	Positive	9.65	47.36	89.70		

Analysis performed using Mann-Whitney test. The SI is considered positive if greater than or equal to 2.

(mean SI, 1.58 ± 0.89 < 3 μg/g dust versus mean SI 2.95 ± 1.82 > 3 μg/g dust, *t* test for equality of means, $p < 0.05$).

Relationship between Parental Atopy, Maternal Sensitization, and *in Utero* Sensitization

We found no relationship between history of atopy or asthma in the parent and the presence of any biomarker in the cord blood, including IgE level and antigen-induced proliferation (data not shown). Maternal total IgE level and cord blood total IgE level weakly correlated ($r = 0.220$; p value < 0.05). In addition, maternal allergen-specific IgE levels to cockroach, *D. farinae*, and mouse did not correlate with cord blood proliferation in response to the same antigen (data not shown). These results suggest that maternal allergic responses to cockroach, dust mite, and mouse proteins may not confer an increased risk for prenatal sensitization to these antigens.

To assess whether antigen-induced T-cell reactivity in the newborn may develop in the absence of antigen-induced T-cell reactivity in the mother, we also compared the same mononuclear cell proliferation assay for sensitization in both mothers and newborns. We found that for cockroach extract, dust mite extract, and tetanus, the presence of proliferation in response to antigen significantly differed between mothers and newborns (Table 4). In many cases, antigen-induced proliferation occurred in cord blood in the absence of antigen-induced proliferation in maternal blood. This finding was more common in response to cockroach (30% of cases) and mouse (24% of cases) extract. These results suggest that *in utero* sensitization can occur in the absence of sensitization within the mother.

DISCUSSION

We found that T-cell reactivity occurred in cord blood in response to multiple indoor antigens, including cockroach and mouse antigen. These findings suggest that *in utero* sensitization to indoor antigens is frequent in a population where asthma morbidity is common (2). T-cell reactivity in response to cockroach and mouse antigen also occurred frequently in a population where environmental exposure to these antigens is prevalent and often ranged well above thresholds previously associated with sensitization (26, 27). Because sensitization to dust mite can occur at levels of exposure less than 2 μg/g dust (28), the low levels measured here may be sufficient for an immune response to develop.

Several observations strongly suggest that the T-cell reactivity we are reporting is antigen-specific. First, the proliferative responses to dust mite protein extracts and highly purified *Der p* 1 and 2 proteins correlated in both cord and maternal blood. Second, T-cell reactivity in cord blood occurred more often to allergens than in response to tetanus toxoid. Finally, cord blood T-cell proliferation occurred significantly more frequently in response to the antigens commonly measured in the apartments than to the rarely detected *D. pteronyssinus* proteins.

The absence of a strong association between T-cell proliferation and allergen-specific IgE levels, as well as the lack of correlation between IL-5 production and T-cell reactivity beyond what we observed after dust mite stimulation, implies that T-cell reactivity is not equivalent to an allergic immune response. An alternative explanation of our results is that the IL-5 upregulation may reflect increased Th2 cytokine production previously reported to occur at the maternal-fetal interface (29). In this milieu, increased placental production of Th2 cytokines may promote *in utero* sensitization by inducing further Th2 proliferation and differentiation as well as upregulating IL-4 mediated MHC II expression (30).

Although the level of mouse antigen measured in settled dust was associated with increased maternal T-cell reactivity in response to mouse extract, this relationship was absent for cockroach and dust mite extract. On the basis of previous reports on levels of dust mite antigen (31), we believe that the bed sample reflects an antigen level that would be correlated with other areas within a pregnant mother's home. However, antigen content in settled dust is only a surrogate for the actual amount of antigen inhaled into the pregnant mother's lungs. It is likely to be an unreliable measure of the biologically effective dose that reaches the fetus. Other unmeasured sources of antigen exposure exist from movable objects such as blankets and exposures outside the home (32). In addition, proteins not measured in our assay (*Der f* 2, *Bla g* 4) may be important. Moreover, host susceptibility (i.e., genetic factors), including MHC class II restriction, contributes to the amount

TABLE 3C. CORRELATION BETWEEN SI AND IL-5 LEVEL*

	(*n*)	Correlation	p Value
PHA	62	−0.232	NS
Cockroach Extract	61	−0.040	NS
D. pteronyssinus Extract	57	0.408	< 0.005
D. farinae Extract	61	0.267	< 0.005
Mouse Extract	59	0.075	NS

TABLE 3D. CORRELATION BETWEEN SI AND IFN-γ LEVEL*

	(*n*)	Correlation	p Value
PHA	63	−0.163	NS
Cockroach Extract	71	0.086	NS
D. pteronyssinus Extract	70	0.241	NS
D. farinae Extract	71	0.263	< 0.001
Mouse Extract	67	0.085	NS

* Analysis performed on raw data using Spearman's rho calculation; p values provided are for two-tailed significance.

TABLE 4. COMPARISON BETWEEN MATERNAL AND CORD BLOOD SI POSITIVITY*

		Cord Blood		
	Maternal Blood	Negative SI	Positive SI	Total
Cockroach	Negative SI	35	40	75
extract	Positive SI	21	37	58
	Total	56	77	133
				$p < 0.05$
D. pteronyssinus	Negative SI	37	13	50
extract	Positive SI	53	20	73
	Total	90	33	123
				$p < 0.001$
D. farinae	Negative SI	28	17	45
extract	Positive SI	49	37	86
	Total	77	54	131
				$p < 0.001$
Mouse	Negative SI	63	30	93
extract	Positive SI	24	15	39
	Total	87	45	132
				p = NS
Tetanus	Negative SI	69	1	70
toxoid	Positive SI	40	3	43
	Total	109	4	113
				$p < 0.001$

* McNemar test of comparison between maternal and cord blood was performed. Two-tailed p values are provided for comparisons in response to each antigen.

of antigen processed and the subsequent development of sensitization. For example, different HLA-DR and DQ genes have been associated with altered dust mite antigen recognition by T cells (33) and dust mite-IgE production (34).

Our observation that cord blood lymphoproliferation can occur more often than maternal lymphoproliferation, and even in its absence, implies that the immune response may be occurring in the placenta. One explanation is that some amount of the relevant antigen, to which the mother is exposed during pregnancy, reaches the placenta where antigen presentation occurs. In support of this hypothesis, antigen-presenting cells (APCs) have been detected in the placenta in increasing numbers during pregnancy. These include dendritic cells that express MHC class II antigens (35) and are efficient in facilitating antigen-induced T-cell proliferative responses (36). Cord blood T cells can be activated via their T-cell receptor (TCR) and express normal levels of CD28, LFA-1, CD40 ligand, and CD25 (37). Cord blood B cells can undergo isotype class switching to IgE and IgG_4 (37, 38). Alternately, the antigens may reach the placenta as IgG-complexed antigens or anti-idiotypic antibodies that have already undergone antigen processing and subsequently sensitize the fetus (39, 40).

Although antigen-induced T-cell proliferation may be a biomarker for antigen exposure, its ability to predict which individuals will develop a subsequent allergic immune response is not known. One group examined the end points of asthma and allergic rhinitis at 12 and at 24 mo of age, even though such diagnoses are difficult at these ages (11), and found they were not associated with increased cord blood proliferation in response to dust mite antigen (20). Another possibility is that Th2 cytokine upregulation in association with T-cell reactivity and/or environmental antigen exposure may predict future atopy. Helminth-specific Th2 upregulation *in utero* was maintained until 10 to 14 mo of age and associated with diminished BCG responses (41), implying that these neonatal patterns may persist and have clinical significance. Prospective study of this cohort will help address the clinical significance of cord blood lymphoproliferation in response to indoor antigens, and help evaluate which biomarkers may identify an individual at increased risk for developing allergic immune responses, including asthma.

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APPENDIX

Comparisons between proliferation in response to *D. pteronyssinus* protein extract (containing *Der p* 1 and *Der p* 2 proteins) and purified *Der p* 1 protein were made by excluding subjects (n = 2 cord blood, n = 1 maternal blood) with a positive SI to *Der p* 2 but not to *Der p* 1 protein. Comparisons between proliferation in response to *D. pteronyssinus* extract and purified *Der p* 2 protein were made by excluding subjects (n = 10 cord blood, n = 6 maternal blood) with a positive SI to *Der p* 1 but not to *Der p* 2 protein.