

Umbilical cord blood mononuclear cell proliferative response to house dust mite does not predict the development of allergic rhinitis and asthma

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Background: Cord blood mononuclear cells (CBMNCs) have been found to proliferate when stimulated with food or inhalant allergens. The significance of this finding is not known.

Objectives: We sought to determine the factors associated with proliferation of CBMNCs when stimulated with house dust mite (HDM) allergen and to determine whether proliferation of CBMNCs is associated with subsequent development of HDM sensitization and development of allergic rhinitis and asthma.

Methods: Cord blood was taken from infants at high risk for having asthma and allergic diseases who were recruited for a primary prevention study in which infants were randomized into the intervention or the control group. CBMNCs were stimulated with HDM allergen and PHA as a positive control. Infants were assessed at 12 and 24 months for health outcome. **Results:** When stimulated with HDM allergen, 29.7% of infants had a stimulation index (SI) of 2 or greater. There were no differences in the levels of cord blood IgE and cotinine, maternal smoking, immediate skin test reactivity, maternal IgE, and maternal exposure to HDM allergen between those with an SI of 2 or greater and those with an SI of less than 2. Infants with an SI of less than 2 had a significantly higher proportion of mothers with a history of asthma. No differences in the incidence of "asthma" and rhinitis without cold at 12 months and cumulative prevalence at 24 months were seen between the 2 groups. None of the infants had skin test reactions to HDM allergen.

Conclusion: The negative association between HDM allergen-induced proliferation of CBMNCs and maternal mite allergen exposure and health outcomes at 12 and 24 months raises the issue of the significance of CBMNC proliferation. (*J Allergy Clin Immunol* 1999;104:317-21.)

Key words: Cord blood, mononuclear cell proliferation, house dust mite, development of asthma and allergic diseases

There have been several studies showing that cord blood mononuclear cells (CBMNCs) proliferated when stimulated with food or inhaled allergens.¹⁻⁶ The significance of this finding is not clear. It is not known whether the mononuclear cells were lymphocytes, and if so, to which functional phenotype they belonged. Moreover, it is not clear whether proliferation of these cells is associated with subsequent development of asthma or other IgE-mediated diseases. In a study involving a small number of infants, Kondo et al⁷ found that infants whose cord blood showed proliferation when stimulated with ovalbumin were at a greater risk of having food-associated atopic dermatitis.

The purpose of this study is 2-fold: (1) to determine whether there is any relationship between proliferation of CBMNCs when stimulated with house dust mite (HDM) allergen and in utero exposure to maternal smoking, maternal history of atopy, and maternal exposure to HDM allergen level and (2) to determine whether proliferation of CBMNCs predicts the subsequent development of atopic diseases in a group of high-risk infants who took part in an intervention study on the primary prevention of asthma.

METHODS

Subjects

Five hundred forty-five high-risk infants, defined as those with at least 1 first-degree relative with asthma or 2 first-degree relatives with other allergic diseases, were identified before birth from prenatal clinics based in Vancouver and Winnipeg. The families were then randomized into either an intervention or a control group. The intervention group received the following measures: HDM avoidance, removal of pets, creation of a smoke-free environment for the infants, breast-feeding for at least 4 months, delayed introduction of solids, and avoidance of major food allergens until 12 months. The details of this study have been reported elsewhere.⁸ Cord blood was obtained from the last 74 infants recruited for the primary prevention study for use in the present investigation.

Data on parental history were gathered by trained nurses by using a modified European Community Survey asthma questionnaire.⁹ Home visits were carried out by research nurses before the birth of the infant to survey the home environment, to collect dust

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Abbreviations used

CBMNC:	Cord blood mononuclear cell
SI:	Stimulation index
HDM:	House dust mite
PRIST:	Paper radioimmunoabsorbent test

samples from various sites in the home for analysis of allergen levels, and to apply intervention measures. A blood sample was also taken from each mother for measurement of total IgE level. To avoid contamination with maternal blood, cord blood samples were obtained by aspiration from the umbilical vein of the placenta. This study had the approval of the ethics committees of both institutions.

Lymphocyte separation and culture

Fifteen milliliters of heparinized cord blood was taken from each infant at birth. Mononuclear cells were isolated by a standard technique of density gradient centrifugation with the use of Histopaque 1077 (Sigma, Inc) after dilution with an equal volume of Tyrode's solution. The cells were washed twice in Tyrode's buffer solution and resuspended in RPMI-1640 (Gibco) supplemented with 10% pooled AB⁺ serum and antibiotics (ie, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin). Cells were cultured at 0.33×10^6 /mL in each well on flat-bottom 10-well plates (Corning, NY), with and without HDM extract, with Der p 1 allergen at 250 AU/mL in a 5% CO₂-humidified incubator for 6 days. PHA was used as a positive control at a concentration of 10 µg/mL. Cell viability was checked before and after culture by exclusion of trypan blue stain, and it was around 95% on both occasions. Optimal allergen concentration in the culture medium had been determined previously by dose-response assays with concentrations of Der p 1 at 0.125, 0.25, 0.5, 1, and 2 µg/mL. Proliferation was detected by the addition of 0.001 µCi/mL tritiated thymidine (Amersham International Ltd) for the last 16 hours of culture. Cells were harvested onto nitrocellulose filter paper and counted in a liquid scintillation β-counter (Beckman Inc). The stimulation index (SI) was calculated as the ratio of tritiated-thymidine uptake in the culture with HDM extract or with PHA to those in the culture without any allergen or mitogen.

Preliminary experiments were carried out comparing the stimulation response of CBMNCs of 9 infants to Der p 1 when serum-free medium (AIM-V, Gibco Life technologies) was used in the cell culture instead of AB⁺ serum. We found that among these 9 samples, an SI of 2 or greater to Der p 1 occurred in 5 samples when AB⁺ serum was used and in only 3 samples when serum-free medium was used. We have decided to use AB⁺ serum in the culture in subsequent experiments.

Measurement of total IgE

Total cord blood IgE and maternal IgE levels were measured by using paper radioimmunoabsorbent test (PRIST) and RIA commercially available kits (Pharmacia Diagnostics, Inc), respectively. Briefly, 0.1 mL of either the standard IgE (0.5 to 100 IgE kU/L in PRIST and 2.0 to 1000 IgE kU/L in RIA) or the serum sample was added to individual test tubes containing a paper disc (PRIST) or sepharose to which anti-IgE was covalently coupled. The tubes were incubated for 3 hours at room temperature. The tubes were then washed 3 times with 2 mL of washing solution that was aspirated completely each time. ¹²⁵I-labeled anti-IgE (0.1 mL) was added to each tube, and the tubes were incubated at room temperature overnight. The next day, the discs were washed 3 times. The radioactivity in each tube was measured in a gamma counter. The activity of the standard or the sample was expressed as a percentage

of the total activity. Each sample was assayed in duplicate. A standard curve was plotted with the percentage values obtained for the standards against the concentration on a linear-log paper. The concentration of the samples was read from the standard curve. The limit of detection of cord blood IgE was 0.05 kU/L.

Measurement of cotinine in cord blood

Cotinine levels in cord blood were determined by means of an RIA.¹⁰ Briefly, 0.1 mL of the cotinine standard (20 to 2000 pg) or 0.1 mL of sample (diluted 1:10) was added to a test tube, followed by 0.1 mL of tritiated cotinine. Anti-cotinine antiserum (0.1 mL) was added to each tube and mixed. The tubes were incubated at 37°C for 60 minutes. After incubation, 0.1 mL of diluted normal rabbit serum was added to each tube, followed by the addition of 0.1 mL of goat anti-rabbit gamma-globulin. The tubes were vortexed and incubated at 4°C overnight and then centrifuged at 1000g for 45 minutes. The supernatant was removed, and the pellet was dissolved in 0.1 mL of 0.1 mol/L NaOH. Ecolume (2.5 mL) was added, and the radioactivity was counted in a β-counter. A standard curve was constructed. The concentration of cotinine in the samples was read from the standard curve and then multiplied by the dilution factor. Each sample was assayed in duplicate.

Mite allergen level in house dust samples

Dust samples were collected from 6 sites: infants' bedroom floors and mattresses, parents' bedroom floors and mattresses, the floors of the most commonly used rooms, and the upholstered furniture in those rooms. A standard protocol was used for dust collection, as described previously.¹¹ The samples were placed in a sealed bag and stored at -20°C until being extracted and analyzed for HDM allergen. The levels were determined by using an ELISA assay with purified mAbs against the group 1 mite allergens of *Dermatophagoides pteronyssinus* and *D farinae* (Der p 1 and Der f 1).¹² The total mite allergen (sum of Der p 1 and Der f 1) was reported as micrograms per gram of dust.¹³

Assessment of health outcome at 12 and 24 months

At 12 and 24 months, each child was examined by a pediatric allergist who was blinded to the randomization. Allergy skin tests were also done by using the prick method with 12 common inhalant and food allergens, including HDM (*D pteronyssinus* and *D farinae*). The health outcomes assessed included "possible asthma," "probable asthma," rhinitis without colds, and atopic dermatitis. "Possible asthma" was defined as at least 2 distinct episodes of cough, each lasting for 2 or more weeks; at least 2 distinct episodes of wheeze, each lasting 1 or more weeks; or, in the absence of a cold, either nocturnal cough (at least once a week) or hyperpnea/exercise-induced cough. "Probable asthma" was defined as at least 2 distinct episodes of cough, each lasting for 2 or more weeks, or at least 2 distinct episodes of wheeze, each lasting for 1 or more weeks, plus at least 1 of the following: nocturnal cough at least once a week in the absence of an apparent cold, hyperpnea-induced or exercise-induced cough or wheeze at any time, or response to treatment with β-agonists and/or anti-inflammatory drugs. Rhinitis without cold was defined as at least 2 episodes of runny nose and sneezing in the absence of a cold.

Statistical analysis

Infants were divided into 2 groups according to their SI to HDM allergen, which was dichotomized at an SI of 2 or greater and less than 2 or an SI of 3 or greater and less than 3. The characteristics between the 2 groups were compared by using chi-square analysis, and the differences in outcome between the 2 groups were tested

TABLE I. Characteristics of study subjects according to SI

	SI <2	SI ≥2
N	52	22
Birth weight (kg)	3.63	3.61
Male, n (%)	26 (50)	11 (50)
Cord blood cotinine level, ng/mL		
Geometric mean (95% CI)	0.45 (0.08-0.95)	0.36 (–0.03-0.91)
Cord blood total IgE, IU/mL		
Geometric mean (95% CI)	0.66 (0.52-0.82)	0.67 (0.48-0.88)
Maternal smoking, n (%)		
NS	37 (71)	13 (59)
EXS	12 (23)	9 (41)
CS	3 (6)	—
Maternal history of asthma, n (%)	35 (67)	9 (41)*
Maternal atopy, n (%)†		
Any allergen	38 (73)	15 (68)
Mite allergen	31 (60)	11 (50)
Maternal total IgE, IU/mL		
Geometric mean (95% CI)	43.7 (24.7-76.6)	25.0 (14.0-44.3)
Maternal mite allergen exposure‡		
Geometric mean (95% CI)	5.4 (3.4-9.2)	5.4 (2.8-10.1)

NS, Nonsmoker; EXS, exsmoker; CS, current smoker.

* $P < .05$ difference between 2 groups as determined by chi-square test.

†Atopy, positive immediate skin test reaction.

‡Mite allergen exposure was calculated as the sum of Der p 1 and Der f 1 levels at 6 sites in the homes during the initial visit. Two families did not have dust samples taken.

with the Fisher exact test. Cord blood IgE and cotinine levels and maternal IgE levels were not normally distributed, and they were transformed into logarithms before analysis. The results of the analyses were similar, irrespective of the level of SI at dichotomy. The results with SI dichotomized at 2 or more and less than 2 are presented below. Nonparametric analyses were also carried out to study the relationship between SI to HDM and cord blood cotinine, cord blood total IgE, maternal total IgE, and maternal exposure to mite allergen level, with all data as continuous variables.

For maternal exposure to HDM allergen, the sum of HDM allergen level (Der p 1 and Der f 1) from samples of dust collected at the onset of the study from 6 sites (ie, parents' bedroom floors and mattresses, infants' bedroom floors and mattresses, the floors of the most commonly used rooms, and the upholstered chairs in the most commonly used rooms) were used.

RESULTS

Of the 74 infants, 52 had an SI to HDM of less than 2, 12 (16.2%) had an SI between 2 and 3, 7 (9.4%) had an SI between 3 and 5, and 3 (4%) had an SI greater than 5. Sixty-eight (92%) infants had an SI to PHA greater than 2. There was no correlation between SI to HDM and SI to PHA. Table I shows the characteristics of the infants according to SI dichotomized at 2.

Twenty-two (29.7%) infants had an SI to HDM allergen of 2 or greater. There were no differences between the 2 groups of infants in their birth weight, percentage of male sex, cord blood total IgE level, and cord blood cotinine level. There was a significantly higher proportion of mothers with a history of asthma in those infants with an SI to HDM of less than 2. The 2 groups were also not different in the proportion of mothers with positive skin reactions to any of the 12 common allergens or to one of the HDM extracts, maternal total IgE levels, and

TABLE II. Health outcome at 12 and 24 months by SI

	SI <2	SI ≥2
Possible asthma		
12 mo	7 (13.5)	3 (13.6)
24 mo	8 (17.0)	3 (13.6)
Probable asthma		
12 mo	4 (7.7)	2 (9.1)
24 mo	6 (12.8)	2 (9.1)
Rhinitis without cold		
12 mo	15 (30.0)	5 (22.7)
24 mo	19 (40.4)	5 (22.7)
Positive skin test to any allergen		
12 mo	9 (18.0)	4 (18.2)
24 mo	11 (22.9)	4 (18.2)

maternal exposure to total HDM allergen or mattress mite allergen levels.

The relationships between CBMNC proliferative responses to HDM allergen and cord blood cotinine, cord blood total IgE level, maternal total IgE, and maternal mite allergen exposure were also analyzed by using SI to HDM as a continuous variable. No correlation was found between SI to HDM and the parameters tested.

Table II shows that there was no difference in the incidence of asthma and rhinitis without colds at 12 months of life between those with an SI of 2 or greater and those with an SI of less than 2. The findings of the cumulative incidence of these parameters at 24 months were similar. None of the infants had a positive skin test reaction to HDM allergen at 12 and 24 months. When the analyses were repeated with SI to HDM dichotomized at 3 or greater and less than 3, the results were not different.

DISCUSSION

In this study we found about 30% of cord blood samples showed a proliferative response when stimulated with HDM allergen. Upham et al¹⁴ reported that a high proportion of CBMNCs proliferated on stimulation with HDM when serum-free medium was used in the cell culture. We were unable to confirm their findings in our preliminary experiments for this study. As a result, we used AB⁺ serum in the cell cultures rather than serum-free medium. We demonstrated that the proliferation of CBMNCs on stimulation with HDM allergen was independent of maternal skin test reactivity to HDM mite allergen, the level of maternal exposure to HDM, and cotinine concentrations in cord blood. Infants with a positive proliferation response to HDM allergen were associated with a lower prevalence of maternal history of asthma.

There have been several reports showing positive proliferative responses by CBMNCs when stimulated with both dietary and inhalant allergens.¹⁻⁶ Most investigators suggested that the proliferative response represents in utero exposure of the fetus to allergens, leading to the priming of T cells.¹⁻⁶ Piccinni et al⁴ demonstrated that although all samples of cord blood responded to stimulation with HDM allergen, only those infants whose mothers had been exposed to the seasonal allergen responded on stimulation to the same seasonal allergen. Jones et al² also found that maternal exposure to birch pollen, which has a discrete season, was an important determinant of CBMNC proliferation when stimulated with birch pollen extract. The proliferative response ratios were higher from samples of cord blood from infants older than 22 weeks' gestation compared with those of less than 22 weeks' gestation. The findings of these studies indicate that environmental inhalant allergens might have crossed the placenta and primed the T cells.

It is not clear whether these allergen-reactive cells in cord blood are maternal in origin and whether these cells are T cells. Holt et al¹ have shown that CBMNCs did not respond to tetanus toxoid, against which most adults respond with proliferation. It is therefore unlikely that these allergen-reactive cells were maternal in origin. More recently, Prescott et al¹⁵ demonstrated that these allergen-specific proliferated cells were likely to be T cells from their cytokine profile. Their finding that these fetal immune responses are likely to be dominated by T_{H2} cytokines is consistent with the recent literature indicating that the immunologic milieu at the feto-maternal interface is skewed toward T_{H2} as an adaptive response to protect the fetus against the toxic effects of IFN- γ .^{16,17} By genotyping these allergen-specific "T-cell" clones, Prescott et al¹⁵ were able to show that these cells were fetal in origin. One should point out that in their study cytokine levels were assayed only semiquantitatively by PCR. Because no markers other than CD4 for selection were done in their study, the results, although suggestive, do not confirm the hypothesis that the proliferating cells were T cells.

Several studies have shown that the types of cytokines produced by fetal mononuclear cells are more important than the type of T cells in the development of atopic disease. Infants whose cord blood showed decreased IFN- γ production when stimulated by PHA were predisposed to subsequent development of atopic diseases.¹⁸⁻²⁰

There is some evidence to suggest that a maternal history of atopy, asthma, or both is more strongly associated with atopic disease in offspring than a paternal history.²¹ Although both parents contribute to genetic transmission of susceptibility, environmental effects on the fetus can only be exerted through the mother. However, in this study we failed to demonstrate the in utero effect of exposure to HDM allergen on CBMNC proliferation. This does not imply that these exposures are unimportant but certainly raises the issue of the significance of mononuclear cell proliferation. We cannot draw any conclusion relating to in utero exposure to maternal smoke because of small sample size.

In a small number of children, Kondo et al⁷ reported that cord blood proliferation stimulated with ovalbumin predicts the subsequent development of food-associated atopic dermatitis at the age of 12 months. We were unable to find any differences between the incidence of "probable and possible asthma" and rhinitis without colds at 12 and 24 months of life in this group of infants. None of the infants had a positive skin test reaction to HDM allergen at 24 months. It is, however, well established that positive skin test responses to inhalant allergens and symptoms associated with sensitization to inhalant allergens do not manifest until after the first 2 years of life.^{22,23} The significance of CBMNC proliferation when stimulated with HDM allergen will not be known until further follow-up studies of these infants are conducted.

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