

Lymphoproliferative responses in cord blood and at one year: no evidence for the effect of *in utero* exposure to dust mite allergens

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Summary

Background Maternal allergen exposure beyond the 22nd week of pregnancy may be important in foetal T cell priming. Allergen-specific cord blood mononuclear cell (CBMC) immunoproliferative responses without corresponding bacterial antigen responses (tetanus toxoid), have been suggested as evidence of *in utero* sensitization.

Objectives To investigate the relationship between lymphoproliferative responses at birth and at 1 year with maternal and 1-year infants house dust mite allergen exposure.

Methods Home visits and dust sampling were performed by the 20th week of pregnancy, immediately after birth, and then at 1 years of age. Der p 1 was assayed using a two-site immunometric ELISA. CBMC immunoproliferative responses (AIM V serum-free medium; 1×10^5 cells/well) were measured for 225 neonates (171 had a high risk of atopy (HR) – both parents skin test positive; 59 had a low risk of atopy (LR) – both parents skin test negative, no history of atopy) by 3H-Thymidine (1 μ Ci/well) incorporation after stimulation in primary culture with phytohaemagglutinin (PHA) (1 μ g/mL), house dust mite [HDM] extract (30 μ g/mL), immunopurified Der p 1 (30 μ g/mL), Tetanus toxoid (TT) (alum free, 30 Lf/mL) or vehicle. Blood was collected from 144 infants at the age of 1 years and stimulated proliferative responses were assessed using the same procedure.

Results PHA-stimulated lymphoproliferative response was significantly lower in HR compared to LR neonates (mean difference 38%, 95% CI 15%–54%; $P = 0.003$); significantly lower proportion of positive CBMC responses to HDM occurred in LR than in HR neonates (30.4% vs. 46.6%; $P = 0.034$). There was no relationship between Der p 1 levels in maternal bed and CBMC immunoproliferative responses, despite the 21 000-fold range of maternal Der p 1 exposure. No significant differences in magnitude, or in proportion of positive responses to any stimulant were observed between the neonates at low, medium or high tertile of allergen exposure. Immunoproliferative responses at birth were not predictive of 1-year PBMC responses. There was no relationship between maternal allergen exposure in pregnancy and 1-year PBMC proliferative responses. However, the proportion of positive proliferative responses at 1 years significantly increased with increasing infant Der p 1 exposure at 1 years.

Conclusion These results indicate that the magnitude of immunoproliferative responses are unrelated to maternal mite allergen exposure and cannot be used as evidence for *in utero* sensitization to inhalant allergens. The immunoproliferative responses at 1 year seem to shift away from the genetically influenced responses at birth towards responses to specific stimulants which correlate with environmental exposure to those specific stimulants. These data support the concept of sensitization to inhalant allergens occurring in early life, but not *in utero*.

Keywords: cord blood mononuclear cells, proliferative responses, allergen, *in utero* exposure

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Introduction

In order to understand the development of atopic diseases and design successful strategies for primary prevention it is essential to establish when the initial imprinting on the immune system occurs [1,2]. In epidemiological studies, early infancy has been identified as a critical period for primary sensitization [2–4] although sensitization to allergens can occur at any age. Immunoproliferation studies, reporting differential stimulation of peripheral blood mononuclear cells (PBMC) from young infants by mitogens and a variety of allergens, have produced data which reinforce the concept of an early sensitization window to allergen exposure [5–8].

It has been suggested that initial priming of the T cell system to environmental allergens may occur before birth [9–17]. Over the past few years a series of immunoproliferation studies reported differential stimulation of cord blood mononuclear cells (CBMC) by mitogens and a variety of allergens [2,9–11]. However, it must be emphasized that lymphoproliferative responses do not necessarily imply sensitization has occurred. Cytokine expression profiling of CBMC immunoproliferative responses has revealed a strong Th2 bias at birth, associated with very low levels of the Th1 cytokine IFN- γ [12–15]. Clonal characterization studies have demonstrated that these responses are neonate-specific and not due to maternal contamination of cord blood [2]. The greater Th2 bias and the lower IFN- γ levels were reported in high-risk vs. low-risk neonates [13–15]. Furthermore, reduced IFN- γ production by antigen-stimulated CBMC has been associated with subsequent development of allergic disease [16,17].

It has been suggested that maternal allergen exposure beyond the 22nd week of pregnancy may play an important role in foetal T cell priming [18]. The allergen-specific differences in CBMC immunoproliferative responses between neonates at high and low risk of developing allergic disease, and the fact that immunoproliferative responses to the bacterial antigen controls (e.g. tetanus toxoid) were not observed, have been suggested as evidence of *in utero* sensitization to inhalant allergens [12,18,19]. This would suggest that the neonate immune system could be committed, rather than merely biased,

towards the development of atopic disease. This has important implications for disease prevention, indicating that any prophylactic intervention(s) need to be implemented before the second and third trimesters of pregnancy.

If CBMC proliferation differences are due to *in utero* sensitization resulting from *in utero* aeroallergen exposure, then their magnitude should reflect maternal exposure over the time concerned. In the current study, we investigated the relationship between lymphoproliferative responses at birth and 1 year with maternal and 1-year infant HDM allergen exposure to evaluate if there is any link between them.

Methods

The National Asthma Campaign Manchester Asthma and Allergy Study (^{NAC}MAAS) is a prospective study which follows the development of asthma and other atopic disorders in a cohort of infants. All mothers were screened for eligibility at 'Booking' antenatal visits (8th–10th week of pregnancy). The study was explained to the parents, the informed consent obtained, and both parents were interviewed for a detailed allergy history and skin tested. Skin tests were performed by prick method and the extracts of the four most common inhalant allergens (*D. pteronyssinus*, cat, dog, mixed grasses) and negative and positive (Histamine dihydrochloride 10 mg/mL) controls were used (Bayer, Elkhart, IN, USA). Test sites were inspected after 15 min and the size of reaction recorded as the mean of the longest and the midpoint orthogonal diameters. Wheal diameter at least 3 mm greater than negative control was regarded as positive response.

The infant cohort

Allocation to study groups for the prospective follow-up Couples who agreed to take part in the study were allocated to risk groups according to their atopic status and a history of allergic disease.

Group A: high-risk, both parents atopic, i.e. skin test positive; no pets Couples were identified as 'high-risk' and eligible for the study if the following criteria were met:

(1) both parents atopic (skin test positive); (2) mother sensitized to at least one of the indoor allergens (dust mite, cat and/or dog). Exclusion criterion was current or future cat and/or dog ownership.

This group was entered into a prospective, prenatally randomised parallel group study investigating whether living in a low allergen environment reduces the risk of primary sensitization and the development of asthma and other atopic diseases. The participants were randomised before 16th week of pregnancy either to the full HDM allergen avoidance (Active group) or a control group. In order to be the most effective in achieving a low allergen environment, an integrated allergen avoidance regime was devised for the Active group of this study. The nature of interventions and the outcomes in terms of reduction in allergen levels are described in detail elsewhere [20].

Group B: high-risk, both parents atopic, i.e. skin test positive; cat and/or dog in home This group included the couples whose child would be at high risk of developing allergic disease, and in addition were cat and/or dog owners. Thus, the only difference compared to Group A was pet ownership.

Group C: low-risk, both parents non-atopic, i.e. skin test negative; no history of atopy This group included the couples whose child would be at low risk of developing allergic disease. Couples were identified as eligible for Group C of the study if the following criteria were met: (1) both parents non-atopic (skin test negative) (2) negative family history of atopic disorders.

Home visits and assessment of environmental allergen exposure After recruitment (between 12th and 16th week of pregnancy) a home visit was made, when dust sampling was performed and an environmental questionnaire completed. All homes were visited again immediately after birth, and then at 1 years of age. Dust samples from the parental bed, neonate's bed, bedroom floor, nursery floor, living room floor and upholstered furniture were collected using a Medivac dust sampler (Medivac Plc, Wilmslow, UK) with air-flow rate 45 L/s through a 355- μ m diameter mesh screen on to a 5- μ m vinyl filter, enabling the collection of fine dust samples. The filters were supported in a plastic dust trap located behind the cleaner nozzle. A 1-m² area of carpet and mattress (upper part) were each sampled for 2 min. Samples were transferred into a preweighed Petri dish, weighed, coded and stored at -35 °C until extraction. One hundred mg of fine dust was extracted with 2 mL borate-buffered saline with 0.1% Tween 20, pH 8.0 (BBS-T). For samples less than 50 mg 1 mL BBS-T was added. Samples between 50 mg and 100 mg were extracted in the proportional amount of BBS-

T (1 : 20). The dust was resuspended using a vortex mixer. Samples were then mixed end over end on an orbital rotator for 2 h at 25 °C before being centrifuged for 20 min at 1100 g at 4 °C. Supernatants were removed and stored at -35 °C for future analysis of allergen content.

ELISA measurement of indoor allergens Der p 1 was assayed using a two-site immunometric ELISA as previously described [21]. The standard used to establish the control curve for Der p 1 assay (UVA 93/02) was considered to contain 2500 ng Der p 1/mL (relative to WHO/IUIS *D. pteronyssinus* standard NIBSC 82/518 which has been estimated to contain 12.5 μ g Der p 1 per ampoule).

Assessment of stimulated proliferative responses

CBMC immunoproliferative responses were measured for 225 neonates (Group A, active, $n = 55$; Group A, control, $n = 49$; Group B, $n = 62$; Group C, $n = 59$). Umbilical cord blood was drawn within 10 min of birth and cell separation commenced within the following 45 min. Cord blood, with 1% heparin as anti-coagulant, was diluted (1 : 1) with AIM V serum-free medium (Gibco BRL, Life Technologies Ltd, Paisley, UK) and twice separated by density gradient centrifugation (Ficoll Paque, Amersham Pharmacia, Uppsala, Sweden). The resulting mononuclear cells were stimulated (triplicate wells) at a density of 1×10^5 cells/well in primary culture (round-bottomed microtitre plates, 37 °C, 5% CO₂). Proliferative responses were assessed by 3H-Thymidine (1 μ Ci/well) incorporation after 3 days stimulation by the mitogen phytohaemagglutinin (PHA, Murex, 1 μ g/mL); or 6 days stimulation by HDM (*D. pteronyssinus*) extract (Bayer, 30 μ g/mL), Der p 1 (affinity purified using a 4C1 monoclonal antibody, 30 μ g/mL), Tetanus toxoid (alum free, Pasteur Merieux 30 Lf/mL) or glycerin vehicle. All stimulants except PHA were analysed to exclude the possibility of endotoxin contamination.

At the clinical follow-up visit at one year of age blood was successfully collected from 144 infants (64 whose CBMC responses had been determined), and stimulated proliferative responses were assessed using an identical procedure. Skin prick tests were performed in all infants with cord blood data (*D. pteronyssinus*, cat, dog, mixed grasses - Bayer). Weal diameter at least 2 mm in diameter greater than negative control was regarded as positive response.

Data analysis

The proliferation and allergen data were found to follow a log-normal distribution; the results are thus reported as geometric means (GM). Der p 1 levels (total allergen

recovered) in maternal mattress were used as the index of maternal exposure during pregnancy, whilst the level in infant mattress represented the measure of exposure of infants at 1 years of age.

There has been considerable variation in reported measures of positivity of stimulated immunoproliferative responses in cord blood. The data were investigated using 6 different measures reported in the literature (stimulation index (SI) ≥ 3 , SI ≥ 2.5 , SI ≥ 2 , $\Delta\text{dpm} \geq 1000$, $\Delta\text{cpm} \geq 1000$ and SI $\geq 2 + \Delta\text{dpm} \geq 1000$). Results for all measures of positivity were very similar, so for ease of presentation only SI ≥ 3 analyses are presented here. Immunoproliferative response data were assessed for normality using Kolmogorov-Smirnoff test in addition to examination of skew and kurtosis statistics. Log-transformed SI values were normally distributed.

The data were analysed using the Statistical Package for Social Sciences (SPSS) version 8.0. Statistical significance was set at a conventional $< 5\%$ level.

Results

Summary responses for each stimulant and group are shown in Table 1 (cord blood) and Table 2 (1 years infants). There were no differences between the groups

(ANOVA) in background (unstimulated) 3H-Thymidine incorporation (log-normalized cpm) after 3 (cord, infants: $P = 0.648, 0.243$) or 6 days ($P = 0.510, 0.466$) in primary culture (data not shown; available on request).

Cord blood mononuclear cells immunoproliferative responses

Significant differences were observed in the pattern of CBMC immunoproliferative responses between neonates at high risk (Groups A and B), and those at low risk (Group C). PHA-stimulated lymphoproliferative response was significantly lower in HR than LR neonates (Fig. 1a: mean difference 38%, 95% CI 15%–54%; $P = 0.003$). A different pattern of response was observed for HDM-stimulated lymphoproliferative response: i.e. a significantly lower proportion of positive CBMC responses to HDM occurred in low-risk neonates compared to those at high risk (Fig 1b and 30.4% vs. 46.6%; $P = 0.034$).

No differences between the groups were found in CBMC responses to immunopurified Der p 1 (Fig. 2a), Tetanus toxoid (Fig. 2b) or vehicle control.

In utero exposure to Der p 1 and CBMC proliferative responses in high-risk infants No effect of maternal HDM

Table 1. Stimulated immunoproliferative responses (SI) in CBMC at birth in four groups of infants

Stimulant	Group			
	Group A (active) ($n = 55$) SI: GM (95%CI) Proportion SI ≥ 3	Group A (control) ($n = 49$) SI: GM (95%CI) Proportion SI ≥ 3	Group B ($n = 62$) SI: GM (95%CI) Proportion SI ≥ 3	Group C ($n = 59$) SI: GM (95%CI) Proportion SI ≥ 3
HDM	2.66 (2.12–3.33) 46.2%	2.62 (2.04–3.37) 37.5%	3.33 (2.69–4.12) 54.1%	2.47 (1.95–3.12) 30.4%
Der p 1	1.37 (0.99–1.90) 21.2%	1.52 (1.18–1.96) 20.8%	2.11 (1.58–2.83) 34.4%	1.40 (1.03–1.90) 26.8%
PHA	367 (288–467) 100%	441 (325–599) 100%	443 (337–582) 100%	665 (512–864) 100%
TT	1.99 (1.68–2.37) 28.8%	2.08 (1.71–2.52) 31.3%	2.63 (2.17–3.20) 36.1%	2.27 (1.85–2.80) 28.6%
Vehicle	0.79 (0.67–0.94) 2.2%	0.86 (0.76–1.04) 0%	0.86 (0.77–0.96) 1.8%	0.88 (0.77–1.02) 3.9%

Group A: high-risk, no pets, active HDM allergen avoidance; Group A: high-risk, no pets, control; Group B: high-risk; cat and/or dog in home; Group C: low-risk. Proportion of positive responses (SI ≥ 3) is also shown.

Table 2. Stimulated immunoproliferative responses (SI) in PBMC at 1 years of age in four groups of infants

Stimulant	Group			
	Group A (active) (<i>n</i> = 32) SI: GM (95%CI) Proportion SI $\geq$ 3	Group A (control) (<i>n</i> = 33) SI: GM (95%CI) Proportion SI $\geq$ 3	Group B (<i>n</i> = 38) SI: GM (95%CI) Proportion SI $\geq$ 3	Group C (<i>n</i> = 27) SI: GM (95%CI) Proportion SI $\geq$ 3
HDM	2.99 (2.25–3.98) 41.9%	3.74 (2.73–5.13) 54.5%	2.92 (2.15–3.97) 45.9%	3.07 (2.29–4.12) 51.9%
Der p 1	0.86 (0.58–1.28) 3.7%	1.00 (0.73–1.36) 7.1%	0.67 (0.52–1.16) 3.0%	0.82 (0.59–1.13) 4.8%
PHA	906 (598–1372) 100%	1358 (837–2203) 100%	899 (596–1757) 100%	888 (563–1402) 100%
TT	18.9 (12.9–27.8) 100%	14.5 (8.4–25.0) 83.3%	16.3 (9.9–26.9) 84.8%	12.2 (7.0–21.0) 84.6%
Vehicle	1.03 (0.80–1.33) 3.4%	0.94 (0.78–1.14) 0%	1.07 (0.82–1.40) 9.4%	1.01 (0.83–1.23) 4.2%

Group A: high-risk, no pets, active house dust mite allergen avoidance; Group A: high-risk, no pets, control; Group B: high-risk; cat and/or dog in home; Group C: low-risk. Proportion of positive responses (SI $\geq$ 3) is also shown.

allergen exposure during second and third trimester of pregnancy upon CBMC immunoproliferative responses was observed using any of a range of approaches to analyse potential relationships.

No correlation was found between log-transformed SI and Der p 1 levels regardless of stimulant (Fig. 3). Although there was 21 000-fold difference in the maternal Der p 1 exposure (0.05–1050 $\mu\text{g/g}$), this did not appear to affect the CBMC responses to either dust mite extract, affinity purified Der p 1, or any other stimulant.

Comparison of log-transformed SI values (ANOVA) revealed no significant difference at birth between the three high-risk groups for any of the stimulants used (Table 1). Similarly, there was no difference in the proportion of positive responses between HR groups for any of the stimulants used (Chi-square test; Table 1), whilst there were major differences in maternal Der p 1 exposure [20].

In order to examine further the effect of maternal Der p 1 exposure upon CBMC responses, proliferation data were stratified with respect to tertiles of allergen exposure during the first and second and third trimesters of pregnancy (Fig. 4). Comparisons were made of all neonates, HR neonates only and LR neonates only. No significant differences in magnitude or proportion of positive responses to any of the stimulants were observed between the infants at low, medium or high level of exposure.

Using logistic regression analysis, positive immunoproliferative responses in HR infants for any of the stimulants were not significantly associated with (1) maternal smoking, (2) number of days before or after due delivery date that the baby was born, (3) maternal age, (4) quarter of the year when child was born or, (5) energy types used at home. Furthermore, no significant association was found between positive immunoproliferative response to any of the stimulants at birth and (1) degree of maternal atopy as assessed by total IgE levels, (2) degree of paternal atopy as assessed by total IgE levels, (3) birth weight and, (4) sex of the neonate. We found no effect of maternal Der p 1 exposure on HDM/PHA, Der p 1/PHA and TT/PHA ratios.

CBMC proliferative responses were not predictive of skin prick test results at 1 years, regardless of stimulant, allergen solution or analysis method, although this may simply reflect the low incidence of positive skin test results at this early age (HR infants: 1.1% mite, 3.7% dog, 4.7% cat, 0% pollen).

PBMC proliferative responses in high-risk infants at 1 years of age

There were no significant differences between the groups in 1-year PBMC responses to any stimulants, including PHA (Table 2). Furthermore, no significant differences were

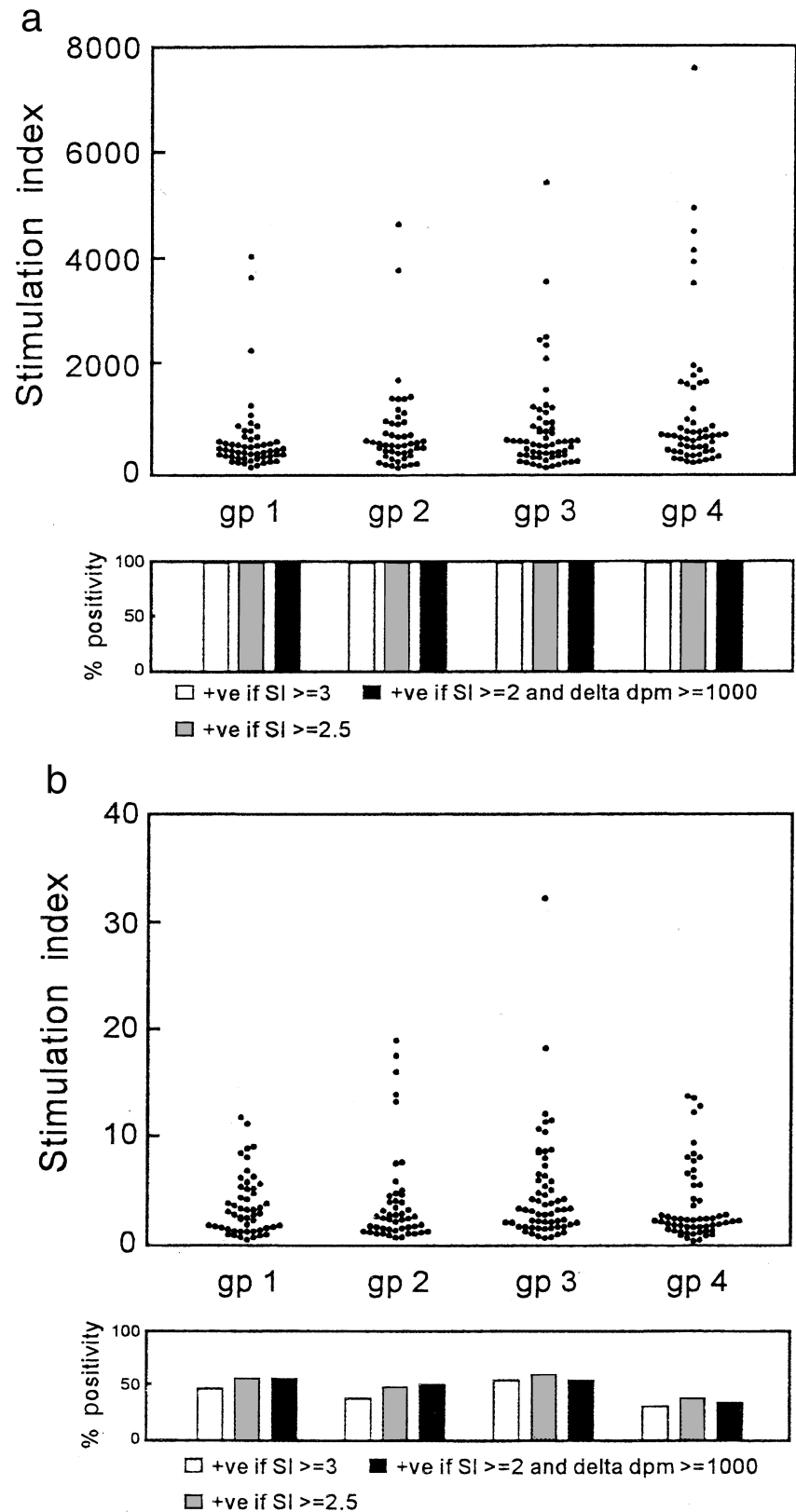


Fig. 1. PHA 1 $\mu\text{g/mL}$ (a) and HDM 30 $\mu\text{g/mL}$ (b) stimulated immunoproliferation in four groups of infants (gp 1: Group A: high-risk, no pets, active HDM allergen avoidance; gp 2: Group A: high-risk, no pets, control; gp 3: Group B: high-risk; cat and/or dog in home; gp 4: Group C: low-risk). Proportion of positive responses (SI ≥ 3 , SI ≥ 2.5 , and SI $\geq 2 + \Delta\text{dpm} \geq 1000$) is also shown.

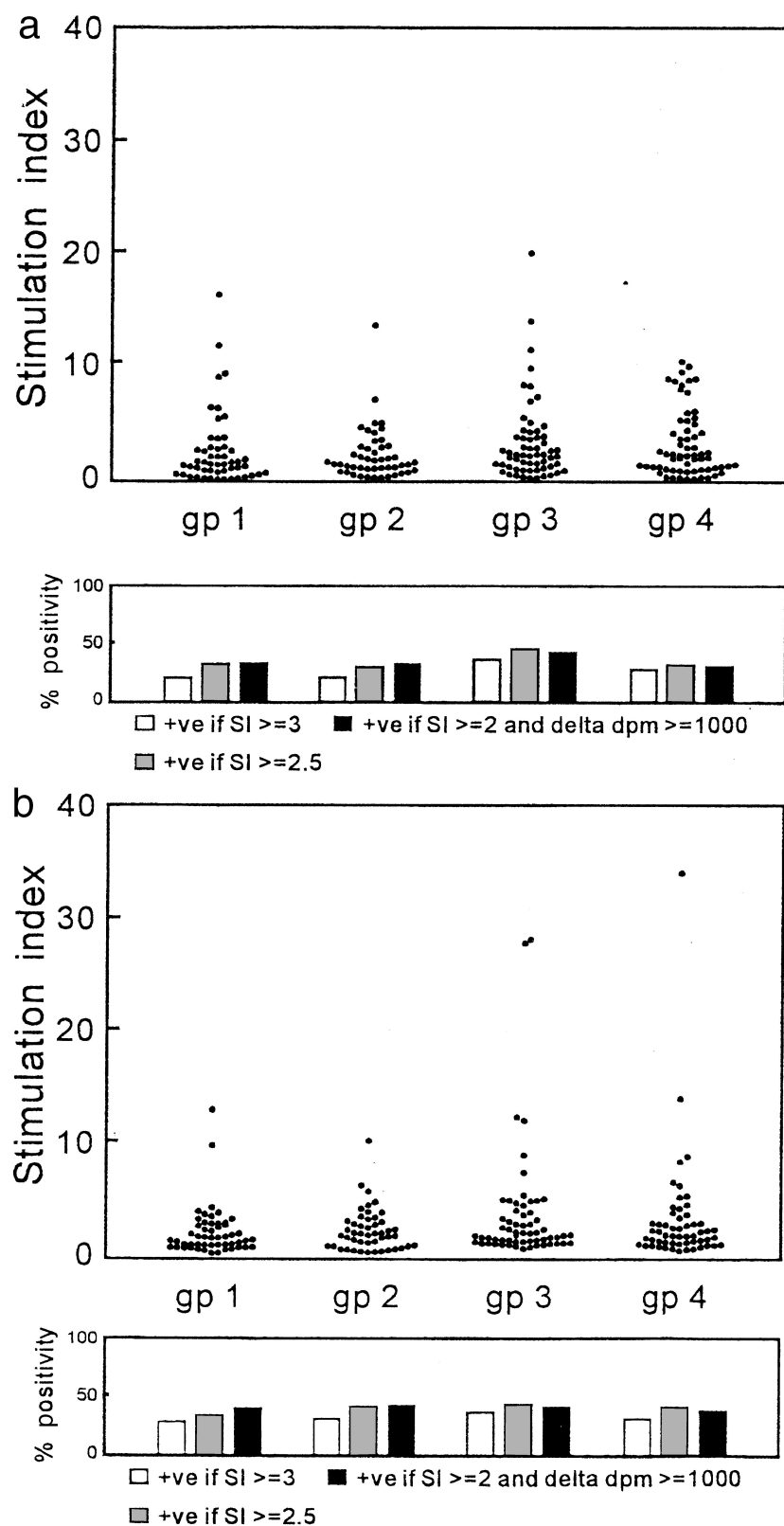


Fig. 2. Der p 1 30 µg/mL (a) and tetanus toxoid 30 Lf/mL (b) stimulated immunoproliferation in four groups of infants (gp 1: Group A: high-risk, no pets, active HDM allergen avoidance; gp 2: Group A: high-risk, no pets, control; gp 3: Group B: high-risk; cat and/or dog in home; gp 4: Group C: low-risk). Proportion of positive responses (SI ≥ 3, SI ≥ 2.5, and SI ≥ 2 + Δdpm ≥ 1000) is also shown.

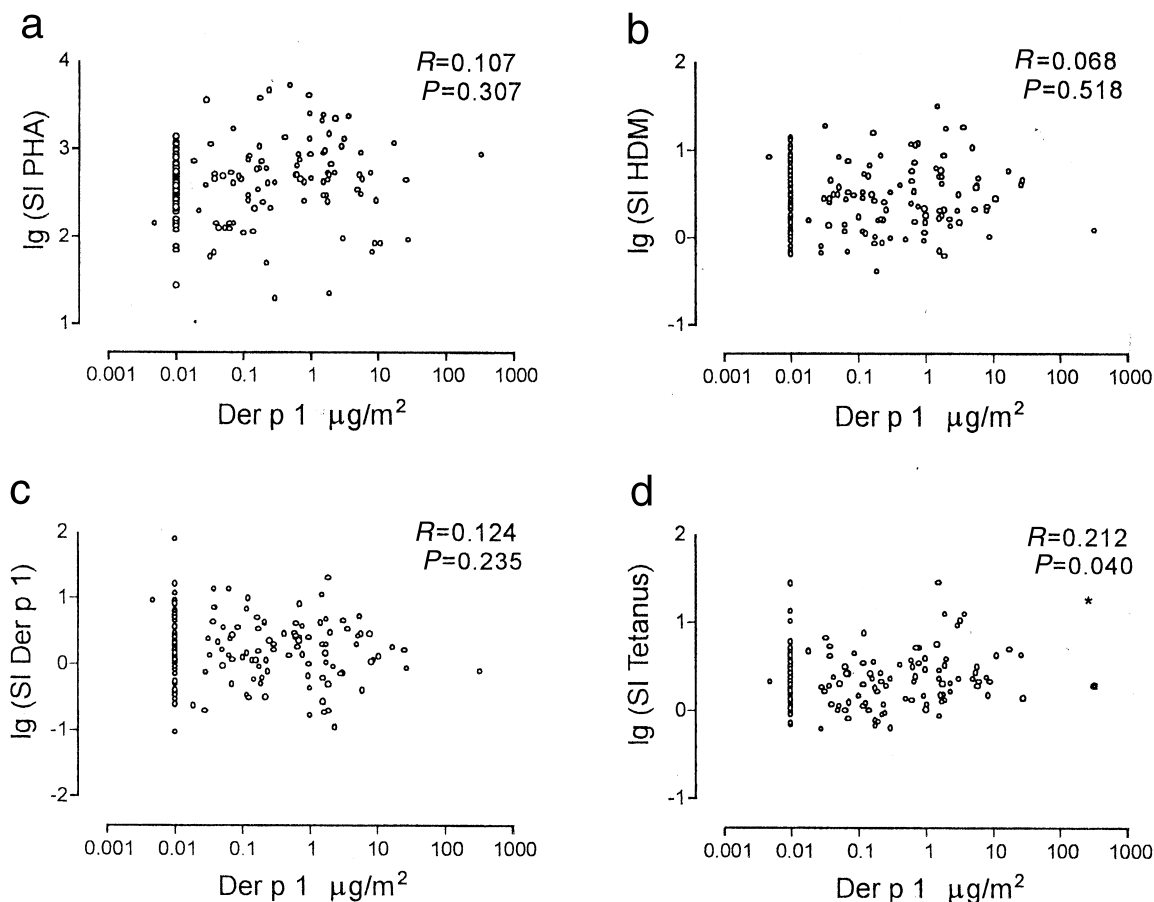


Fig. 3. Stimulated CBMC proliferation results expressed in stimulation indices to PHA (a), HDM (b), immunopurified Der p 1 (c) and tetanus toxoid (d) in relation to maternal Der p 1 exposure during second and third trimester of pregnancy.

found between HR and LR infants in magnitude of PBMC responses or the proportion of positive responses to any of the stimulants (Table 2).

Prediction of 1 years PBMC responses from CBMC responses and maternal exposure Immunoproliferative responses at birth were not related to 1-year PBMC responses using the 64 cases where longitudinal data was available:

1 There was no significant correlation between proliferation at birth and 1 years, except for a very weak negative correlation for Der p 1 (data not shown, available on request).

2 Positive response at birth did not predict positivity at one year of age (e.g. HDM – 54% of positive at birth were negative at 1 years, 40% of negative at birth were positive at 1 years).

There was no correlation between maternal Der p 1 exposure before birth and one year proliferative responses to any stimulant.

Infant dust mite allergen exposure and immunoproliferative responses at age 1 years No correlation was found between SIs and allergen levels, regardless of stimulant or infant group. To further examine the effect of allergen exposure upon PBMC responses, proliferation data were stratified with respect to tertiles of infant mite allergen exposure (mattress Der p 1-total allergen). There were significant differences in the proportion of positive responses between infants exposed to low, medium and high Der p 1 levels for HDM, but not other stimulants (35.4%, 53.8% and 60.7% in low, medium and high exposure group, respectively, $P = 0.024$; Fig. 5).

Discussion

In the current study we demonstrate for the first time that the level of maternal dust mite allergen exposure during pregnancy did not affect CBMC immunoproliferation. Regardless of the approach to the analysis, the data resembled a random distribution, with no impact of

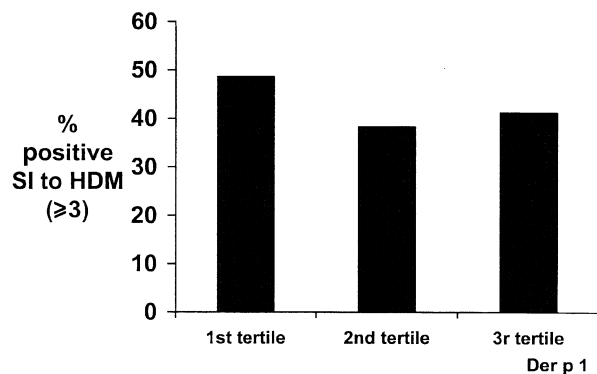


Fig. 4. Proportion of positive CBMC immunoproliferative responses to dust mite extract ($SI \geq 3$) in low, medium and high tertile of maternal dust mite allergen exposure during pregnancy.

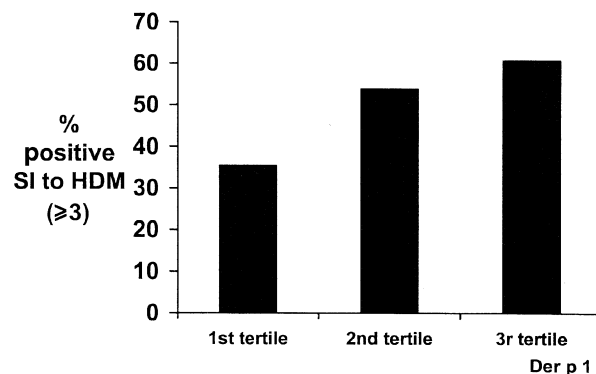


Fig. 5. Proportion of positive PBMC immunoproliferative responses to dust mite extract ($SI \geq 3$) at 1 years of age in low, medium and high tertile of infant dust mite allergen exposure at the age 1 years.

maternal mite allergen load. Thus, if *in utero* sensitization to mites occurred, it was not detectable by CBMC immunoproliferation.

Furthermore, neonate CBMC immunoproliferation was not limited to allergen stimulation – we observed naïve responses to tetanus toxoid equivalent to Der p 1 (i.e. 26.3% and 31.3% of CBMC responded to Der p 1 and TT, respectively). This result contrasts sharply with the recent findings by Prescott *et al.* who reported positive responses to allergens (dust mite, immunopurified Der p 1, Fel d 1, β -lactoglobulin, ovalbumin (OVA) and rye grass) in the absence of response to TT, and consequently concluded that sensitization to allergens had already occurred *in utero* [2]. The difference in observations may be due to the concentration of TT used in two studies, as the concentration used by Prescott *et al.* was 60 times lower than the optimum established in our lab (0.5 vs. 30 Lf/mL) [2]. Our data suggest that CBMC immunoproliferative responses are not limited to allergens. Furthermore, as exposure to TT is unlikely during pregnancy, it seems plausible that this is a non-specific response reflecting how many proteins are present for the T cells to react to (e.g. HDM protein mixture elicited considerably greater response than immunopurified Der p 1).

Indoor pollution (home heating and smoking) and birth weight and gestational age, which could be linked with immune system maturity and might affect *in utero* sensitization had no effect on CBMC responses.

It has to be emphasized that the fact that proliferation is not linked to *in utero* exposure to mite allergens cannot be taken as evidence that raised/lowered CBMC immunoproliferation does not predispose neonates to subsequent development of atopy.

As others have previously reported [2,5,10], we have observed differences in CBMC proliferation between infants at high and low risk of developing atopy (low risk infants having higher mitogenic immune responses, yet

lower responses to mite allergen extract). This different pattern of response did not appear to be due to environmental mite allergen exposure. There are a limited number of alternatives to explain these differences. A difference in foetal environment because of the mother's allergic disease cannot be excluded, but there was little evidence for this, as degree of parental atopy (assessed by the total serum IgE level) had no effect on lymphoproliferation. Maternal medication (e.g. steroids) or other environmental factors (e.g. pet ownership or the type of fuel used in home) may have an effect on uterine environment, but we did not find the evidence to support this. A further possibility would be a genetic difference in regulation of cell profile. There is evidence that different cell profiles exist in high-risk and low-risk neonates [5,22,23], and this could cause differences in response when mitogenic stimulation is compared to antigenic, as different mechanisms are involved. A strong suppressive effect of immature $CD4^+$ cord blood T cells on T helper function has been demonstrated [24]. It can be hypothesized that the genetic risk of atopy is associated with delayed postnatal maturation of T cell competence [25]. It is possible that individuals who subsequently develop atopy may inherit a greater number or different profile of T cell receptors which react with allergens (thus higher responses occur), whilst at the same time there may be fewer cells in total. Alternatively, there may be signal transduction differences with differential expression of the cells and proteins involved in mitogenic and antigenic responses [26,27].

It has been suggested that the subpopulation of high-risk neonates that develop atopic disease by 1 years of age are more likely to respond to dust mite, with their responses being significantly above low-risk and high-risk neonates that do not develop atopy [5]. In the current study we found no evidence that the PBMC response at 1 years of age can

be predicted from CBMC lymphoproliferative responses at birth. Furthermore, dust mite allergen exposure before birth did not predict 1-year PBMC responses. These observations do not support the concept of *in utero* sensitization [1,28] and they provide an interesting parallel to observations by other groups that cord IgE levels, although variable at birth, are not predictive of subsequent values [29].

There was no evidence that parental atopic status was predictive of 1-year PBMC responses, indicating that genetic factors are of less relevance to the magnitude of PBMC responses at one year than CBMC responses at birth. This observation also reinforces the impression that 1-year responses are 'uncoupled' from CBMC responses.

The results of the current study suggest that 1-year PBMC mite allergen specific immunoproliferative responses were greater with greater exposure to dust mites at one year. This would be compatible with the hypothesis of early sensitization occurring (or beginning to occur) within the first year of life. Together with the lack of responses at birth, this observation would not support the hypothesis of early sensitization occurring *in utero*.

The best evidence of *in utero* sensitization to inhalant allergens would be a relationship between response and maternal exposure. It had been assumed that responses in high-risk infants reflected exposure, but our data would not support this. Furthermore, an effect was seen at 1 years demonstrating that the lymphoproliferation system is capable of responding to mite allergen exposure.

In conclusion, the results of the current study may indicate that the magnitude of immunoproliferative responses cannot be used as evidence for *in utero* sensitization to inhalant allergens, but suggests that there are differences at birth between neonates at high and low risk of atopy, perhaps caused by genetic differences. It is possible that these are due to mechanistic differences between high-risk and low-risk neonates, both in antigenic and in mitogenic responses, which predispose the former to atopy development, especially if environmental conditions in early life are unfavourable. The immunoproliferative responses at 1 years seem to shift away from aeroallergen exposure-independent (perhaps genetically influenced) responses at birth towards responses to specific stimulants which correlate with environmental exposure to those specific stimulants. These data support the concept of sensitization to inhalant allergens occurring in early life, but not *in utero*.

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