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Developing Patterns of T Cell Memory to Environmental Allergens in the First Two Years of Life

Abstract

Several recent studies have demonstrated cord blood mononuclear cell (CBMC) proliferation in response to food and inhalant allergens, suggesting that initial T-cell-priming may occur in utero. The findings below from an ongoing prospective study on 60 subjects provide initial information on the nature of accompanying T cell cytokine responses. We demonstrate CBMC proliferation following culture with house dust mite and ovalbumin (OVA) in 47 and 42% of subjects, respectively, compared to an overall rate of 3% for tetanus toxoid; the frequencies of these responses were comparable in neonates with and without atopic family history (FH). With the exception of IL-10, analysis of cytokine responses in allergen-stimulated cultures of CBMCs required the use of semiquantitative RT-PCR, which revealed low-level IL-4 and/or IL-5 mRNA production, in particular a 50% IL-5 response rate to OVA in FH-positive neonates. IFN- γ responses were less frequent and required higher PCR cycle numbers for detection. Preliminary analysis of culture supernatants from a subgroup of CBMCs indicate high-level allergen-specific IL-10 responses in both FH-negative and -positive subjects, detectable by ELISA. Parallel PCR studies on MCs from 27 children (mean age 18 months) indicated a clear segregation at this age on the basis of FH, with Th0-like or mixed Th1/Th2 responses (IL-5 plus IFN- γ) which were mainly restricted to the FH-positive group.

Key Words

Allergy
Th1/Th2
Cytokines
Tolerance/suppression
Cord blood

Introduction

Atopic disease in human adults is associated with the expression of aeroallergen-specific Th memory which is characterised by production of IL-4 and IL-5 [1, 2], analogous to the murine Th2 cytokine pattern [3]. In contrast, non-atopic adults express aeroallergen-specific Th memory which is polarised towards the murine Th1 pattern [1, 2]. The overall prevalence of Th2-dependent allergy to inhalants amongst adults typically is in the range of 30-35% in the developed countries [4], whereas allergy to foods is comparatively rare, which is consistent with the operation of distinct

mechanisms for the regulation of Th cell memory development against allergens presented via the gastro-intestinal and respiratory mucosae.

Recent reports from a number of laboratories have demonstrated that the initial priming of the T cell system against both inhalant and food allergens occurs during the perinatal period [5-9], and respective Th memory populations are subsequently shaped by allergen-driven T cell selection/deletion during early childhood [10]. These studies are essentially based on the use of lymphoproliferation assays, and only limited information is available on relevant T cell cytokine responses over this age range. In particular, despite in-

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direct evidence of low-level fetal IgE synthesis [11] and of spontaneous production of high levels of Th2 cytokines by the fetoplacental unit throughout gestation [12], there is as yet no published evidence of allergen-specific Th2 cytokine responses by human neonatal lymphocytes.

The present study reveals that a high proportion of cord blood mononuclear cells (CBMCs) produce mRNA specific for IL-5 following in vitro stimulation, particularly CBMCs from family-history (FH)-positive subjects exposed to the food allergen ovalbumin (OVA); moreover, by 18 months of age, the response pattern amongst FH-positive children had consolidated into a Th0-like cytokine profile, while at the population level, the FH-negative group remained unresponsive.

Methods and Materials

Subjects

All subjects were classified regarding the occurrence of atopy in first-degree relatives employing a standard questionnaire [13]. Cord blood was collected from healthy term newborns with ($n=46$) and without ($n=14$) an FH of allergy. A group of infants (mean age 17.9 months) were studied as an age comparison.

Lymphoproliferation

CBMCs and peripheral blood MCs (PBMCs) which had been cryopreserved at collection were resuspended at 1×10^6 viable cells/ml in AIM V serum-free medium for standard microplate assays as previously described [9] and cultured in the presence of extracts of *Derma-tophagoides pteronyssinus* (CSL Ltd., Melbourne, Australia) at a final concentration of 10 $\mu\text{g/ml}$ and OVA (Sigma, St. Louis, Mo., USA) at a final concentration of 100 $\mu\text{g/ml}$. A positive response was defined as delta dpm (counts in stimulated wells - controls) $>1,000$ together with a stimulation ratio (stimulated well/control) >2 .

Polymerase Chain Reaction

For cytokine analyses, aliquots of MC at $1 \times 10^6/\text{ml}$ were cultured for 24 h at 37°C with 5% CO_2 in medium containing house dust mite (10 $\mu\text{g/ml}$) or OVA (100 $\mu\text{g/ml}$); parallel background control cultures contained cells in medium alone. RNA extraction and cDNA synthesis employed standard techniques. PCR methodology and subsequent quantitation of PCR products by Southern blotting were based on procedures described previously [14]. Cycle analyses were performed for β -actin and each of the cytokines for each set of samples to ensure that detection levels had been reached and that the reaction remained in the linear phase. For cord blood samples, this was 47 cycles for IFN- γ , 28 cycles for β -actin and 43 cycles for IL-5; for infants, this was 45 cycles for IFN- γ , 40 cycles for IL-5 and 30 cycles for β -actin. Detection and semiquantitative analysis of PCR products were performed by dot blotting, hybridisation and chemiluminescence. The intensity of each dot was then determined using computerised densitometry, and the results were expressed as a ratio of cytokine to β -actin density after subtraction of backgrounds. Non-parametric analysis (Mann-Whitney, two tailed) was performed, using the program InStat 2.0 (GraphPad Software, San Diego, Calif., USA).

Cytokine ELISA

A standard ELISA assay which is sensitive to 150 pg/ml IL-10 was performed for the detection of IL-10. The assay was employed on 24-hour supernatants from the same cultures employed above for PCR studies. Previous experience with IL-4, IL-5 and IFN- γ indicates that the levels of these cytokines produced by neonatal MCs are below the levels of detection by conventional ELISA.

Results

House dust mite and OVA were selected as archetypal airborne and food allergens, respectively. The data from cord blood in figure 1 show that lymphoproliferative responses to both classes of allergen are present at birth, with 47% of babies showing positive responses to house dust mite and 42% responding to OVA. This responsiveness was not related to allergic FH, as a similar proportion of FH-positive and FH-negative babies responded to each of these allergens. Although FH-positive babies tended to have slightly higher responses than FH-negative babies (expressed in fig. 1 as delta dpm), this difference was not statistically significant. In contrast to these responses to allergen, responses to tetanus toxoid were rare (3% of all babies) and of low magnitude.

As shown in figure 2, a proportion of babies in both FH-positive and FH-negative groups showed an increase in IL-5 and IFN- γ -specific mRNA production in response to allergen stimulation at birth; IL-4 responses were also observed in both groups at a slightly lower frequency (data not shown). However, at the population level, the only response which attained statistical significance was the production of IL-5 mRNA induced by OVA in the FH-positive group ($p=0.036$). The mean IFN- γ mRNA responses to the allergens were not significantly different from backgrounds; it should also be noted that in order to detect an IFN- γ signal, it was necessary to employ very high cycle numbers (47) in the PCR, suggesting an extremely low precursor frequency of specific cells producing this cytokine (further discussion below).

By the age of 18 months, clear differences were evident between FH-positive and FH-negative subjects (fig. 2b). Notably, statistically significant IL-5 and IFN- γ responses to both allergens were observed in the FH-positive group, whereas the median responses in the FH-negative group were not significantly above background.

Preliminary studies on culture supernatants from OVA- or PHA-stimulated CBMCs from 12 FH-positive and 12 FH-negative subjects (table 1) revealed frequent high-level IL-10 responses in both groups.

Fig. 1. Lymphoproliferative responses of CBMC from allergic FH-negative (H-) and positive (H+) newborns after 7 days of culture with crude house dust mite (HDM), OVA or tetanus toxoid (TT). Individual responses and group means are shown.

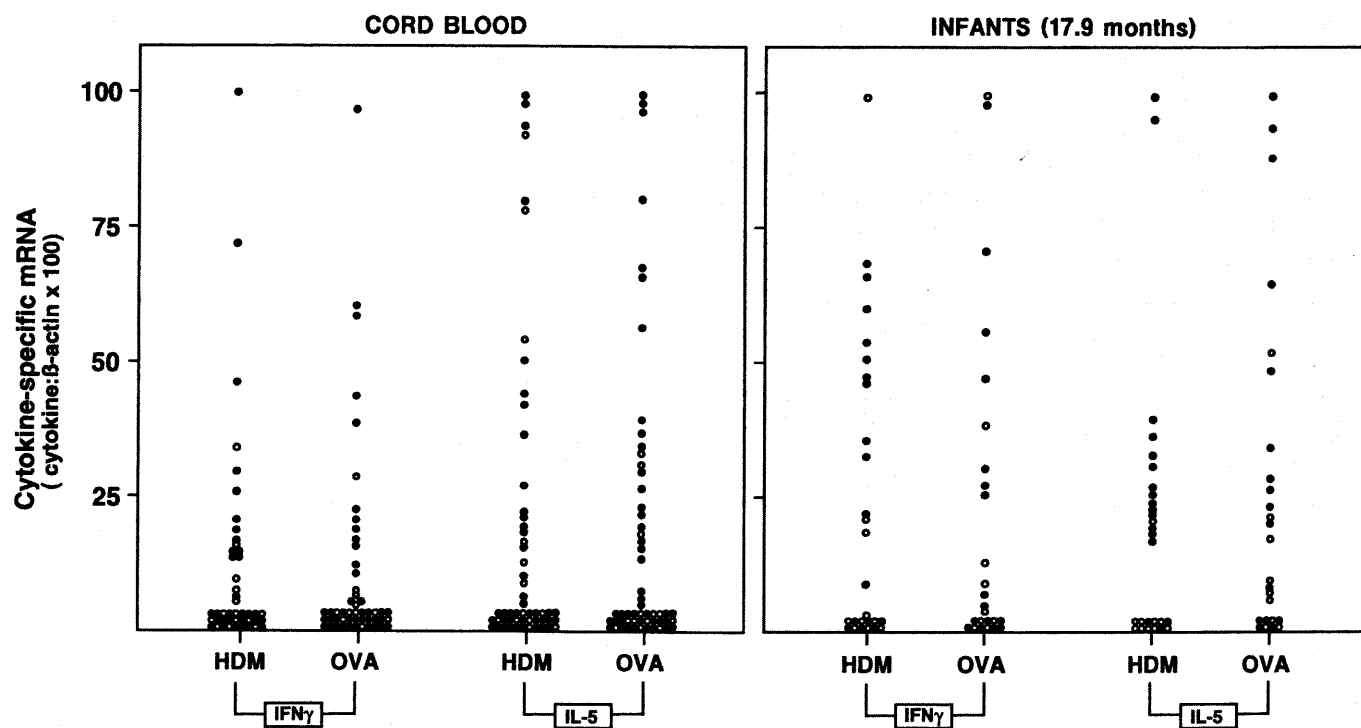
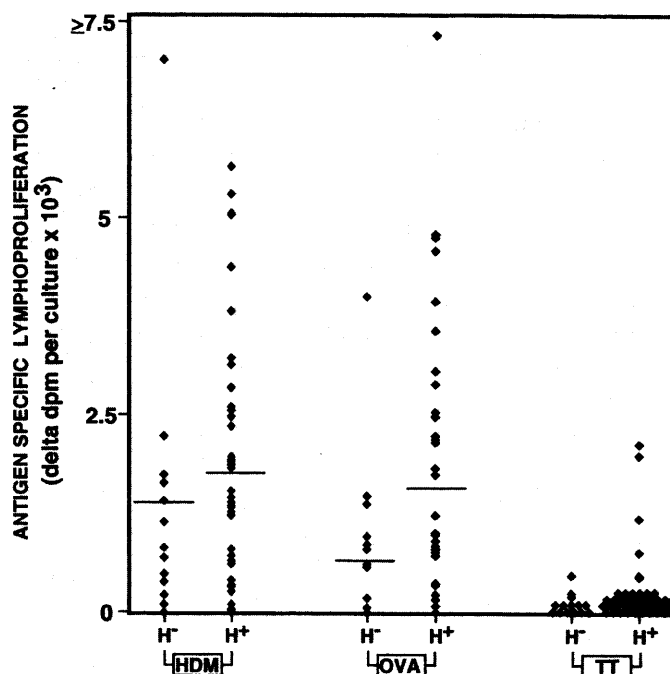


Fig. 2. IL-5 and IFN γ -specific mRNA responses of CBMCs (neonates) and PBMCs (at mean age 17.9 months) to crude house dust mite (HDM) and OVA. Cytokine mRNA is expressed as a ratio to β -actin mRNA after subtraction of respective background control levels. Allergic FH-negative babies are shown as open circles and FH-positive babies as closed circles.

Discussion

The cytokine mRNA responses of CBMCs to both food and aeroallergen detected in this study lend further weight to earlier suggestions that the neonatal immune system is commonly primed to these classes of antigen in utero. The finding that statistically significant IL-5 responses in CBMCs were restricted to the FH-positive group may indicate that the polarisation of allergen-specific T cell responses has already been initiated at birth in subjects genetically at high risk of atopy, and this impression is reinforced by further consolidation of IL-5 responses in FH-positive infants by 18 months of age. The present data on IL-5 and IFN- γ responses are based solely on the production of cytokine-specific mRNA, and further studies are required to ascertain whether this mRNA is translated into bioactive cytokine. However, the high frequency of unequivocal high-level allergen-specific production of IL-10 protein illustrated in table 1 argues strongly that these represent genuine immune responses.

From these data, it also appears likely that there is a significant overlap between the cytokine responses of FH-negative and FH-positive subjects at the population level. In this context, it should be noted that the detection of IFN- γ signals in the CBMC samples required the use of much higher PCR cycle numbers than was required for IL-5; this pattern is the reverse of that observed employing allergen-stimulated adult PBMC samples tested in the same detection system (data not shown), and indicates a generalised skewing of the fetal allergen-specific T cell responses away from Th1. It is also pertinent to note that IL-10 is a potent inhibitor of IFN- γ production by human T cells [15].

These findings are consistent with the growing literature [e.g. see ref. 12] which suggests that the fetal cytokine milieu is constitutively polarised towards Th2 in order to protect the fetoplacental unit from the toxic effects of Th1 cytokines such as IFN- γ , a process in which IL-10 is proposed to play a key role [16]. It is possible that this Th2 polarisation process may be more profound and long lasting in children

Table 1. Allergen-specific and polyclonal IL-10 responses of CBMCs

FH negative				FH positive			
subject No.	control	OVA	PHA	subject No.	control	OVA	PHA
03	<	420	2,300	04	770	4,900	1,450
10	<	2,000	3,600	05	680	2,450	3,600
13	<	1,200	580	06	<	410	1,450
17	420	3,100	2,200	09	<	470	2,600
21	<	<	1,600	12	1,640	1,750	1,100
22	<	<	300	14	230	4,300	2,400
23	1,400	7,000	6,400	19	<	650	1,000
33	2,000	6,800	3,000	24	165	2,500	2,500
39	<	<	2,100	30	3,000	4,300	2,000
47	1,400	3,700	3,200	32	15,500	22,000	8,600
53	<	5,800	3,900	51	680	2,700	3,000
60	<	570	2,000	56	250	180	2,500

Data shown are pg/ml IL-10 from 24-hour culture supernatants.
< = Below detection limit.

with the atopic genotype, a suggestion that is consistent with reports from several laboratories [reviewed in ref. 17] indicating depressed IFN- γ -secreting capacity in PBMCs from FH-positive infants. If this is the case, the initial selection of Th memory cells specific for environmental allergens encountered in late fetal or early postnatal life would favour the Th2 phenotype, possibly resulting in the differences between the IL-5 responses of FH-positive and -negative subjects shown in figure 2. In more general terms, this may also explain the existence of the high-risk 'window' for primary allergic sensitisation during infancy [18] which is considerably more marked in FH-positive subjects.

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References

- 1 Wierenga EA, Snoek M, de Groot C, Chrétien I, Bos JD, Jansen HM, Kapsenberg ML: Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651-4656.
- 2 Romagnani S: Induction of TH1 and TH2 responses: A key role for the 'natural' immune response? *Immunol Today* 1992;13:379-381.
- 3 Mosmann TR, Coffman RL: TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145-173.
- 4 Cline MG, Burrows B: Distribution of allergy in a population sample residing in Tucson, Arizona. *Thorax* 1989;44:425-431.
- 5 Kondo N, Kobayashi Y, Shinoda S, Kasahara K, Kameyama T, Iwasa S, Orii T: Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992;67:1003-1007.

- 6 Piccini M-P, Mecacci F, Sampognaro S, Manetti R, Parronchi P, Maggi E, Romagnani S: Aero-allergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993;102:301-303.
- 7 Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO: Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994;24:423-430.
- 8 Piastra M, Stabile A, Fioravanti G, Castagnola M, Pani G, Ria F: Cord blood mononuclear cell responsiveness to beta-lactoglobulin: T-cell activity in 'atopy-prone' and 'non-atopy-prone' newborns. *Int Arch Allergy Immunol* 1994;104:358-365.
- 9 Upham JW, Holt BJ, Baron-Hay MJ, Yabuhara A, Hales BJ, Thomas WR, Loh RKS, O'Keeffe P, Palmer P, Le Souef P, Sly PD, Burton PR, Robinson BWS, Holt PG: Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: Covert responses are unmasked by serum-free medium. *Clin Exp Allergy* 1995;25:634-642.
- 10 Holt PG, O'Keeffe PO, Holt BJ, Upham JW, Baron-Hay MJ, Suphioglu C, Knox B, Stewart GA, Thomas WR, Sly PD: T-cell 'priming' against environmental allergens in human neonates: Sequential deletion of food antigen specificities during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995;6:85-90.
- 11 Croner S, Kjellman NIM: Development of atopic disease in relation to family history and cord blood IgE levels. Eleven year follow-up in 1654 children. *Pediatr Allergy Immunol* 1990;1:14-20.
- 12 Wegmann TG, Lin H, Guilbert L, Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993;14:353-356.
- 13 Holt PG, Clough JB, Holt BJ, Baron-Hay MJ, Rose AH, Robinson BWS, Thomas WR: Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992;22:1093-1099.
- 14 Doi S, Gemou-Engesaeth V, Kay AB, Corrigan CJ: Polymerase chain reaction quantification of cytokine messenger RNA expression in peripheral blood mononuclear cells of patients with acute exacerbations of asthma: Effect of glucocorticoid therapy. *Clin Exp Allergy* 1994;24:854-867.
- 15 D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Ku M, Trinchieri G: Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ -production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 1993;178:1041-1048.
- 16 Mosmann TR, Sad S: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996;17:138-146.
- 17 Holt PG: Primary allergic sensitisation to environmental antigens: Perinatal T-cell priming as a determinant of responder phenotype in adulthood. *J Exp Med* 1996;183:1297-1301.
- 18 Holt PG, McMenamin C, Nelson D: Primary sensitisation to inhalant allergens during infancy. *Pediatr Allergy Immunol* 1990;1:3-13.