

Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents

E. A. MILES, J. A. WARNER, A. C. JONES, B. M. COLWELL, T. N. BRYANT* and J. O. Warner

Child Health and *Department of Medical Statistics and Computing, University of Southampton, UK

Summary

Background Raised peripheral blood mononuclear cells (PBMCs) proliferative responses to food allergens have been demonstrated in children with established atopic dermatitis.

Objective In this report we investigate the PBMC proliferative responses to inhalant and food allergens from babies at birth, 6 months and 1 year of age, born to atopic and non-atopic parents.

Methods PBMCs, separated by density gradient centrifugation, were cultured for 6 days with autologous plasma and a range of allergens (house dust mite [HDM], cat, grass pollen, tree pollen, betalactoglobulin and ovalbumin). Proliferative responses were measured by the uptake of [^{3} H] thymidine added for the final 18 h of culture.

Results At birth, infants born to atopic parents who developed allergic disease by 1 year of age had significantly more positive responses (stimulation index ≥ 2 with a value of ≥ 1000 cpm above background) to HDM ($P = 0.0097$), betalactoglobulin ($P = 0.0166$) and ovalbumin ($P = 0.0035$) than newborns who did not develop allergy. Infants who developed allergy also had significantly more positive responses to HDM ($P = 0.03$) and ovalbumin ($P = 0.0057$) than babies, born to non-atopic parents, who did not develop allergies. At 6 months of age a significant fall in response to HDM ($P = 0.003$) and cat fur extract ($P = 0.006$) was seen in infants who developed allergic disease by 1 year of age. A similar pattern was seen for proliferative responses to betalactoglobulin and ovalbumin ($P = 0.0006$, $P = 0.004$). Conversely, proliferations to grass and tree pollen extracts increased at 6 months ($P = 0.04$, $P = \text{NS}$) and 1 year ($P = \text{NS}$, $P = 0.01$) compared with birth which was significant for infants who did not develop allergic disease.

Conclusion Proliferative responses to seasonal allergens increased over the first year of life whilst those to perennial allergens, both inhalant and food, fell. This suggests either the induction of a systemic immune tolerance by perennial exposure to antigens or movement of sensitized cells to target organs where allergen exposure occurs. This process may be independent of the development allergic disease.

Keywords: allergy, allergen, cord blood, inflammation, PBMC proliferative response

Clinical and Experimental Allergy, Vol. 26, pp. 780–788. Submitted 19 September 1995; revised 28 November 1995; accepted 8 February 1996.

Introduction

Raised peripheral blood mononuclear cell (PBMC) proliferative responses to food antigens have been demonstrated

in children with food-sensitized atopic eczema [1]. The responding cells are predominantly CD4⁺ T cells and it has been suggested that these cells played a role in IgE-mediated hypersensitivity in patients with atopic eczema [2].

We have shown that infants who subsequently develop food-sensitive eczema have a higher PBMC proliferative

Correspondence: Dr E. A. Miles, Child Health, Southampton General Hospital, Tremona Road, Southampton SO16 6YD UK.

response and lower interferon-gamma generation at birth in response to stimulation by betalactoglobulin from cows' milk and ovalbumin from hens' egg than children with non-food sensitive eczema or those with no eczema [3]. This is similar to the results of another study looking at the PBMC proliferative response to bovine serum albumin and ovalbumin in 37 cord blood samples which indicated that raised PBMC proliferative responses were a more accurate predictor of subsequent allergic disease than raised cord blood IgE or a family history of allergy [4].

Rawle *et al.* showed strong PBMC proliferative responses to the major house dust mite allergen (Der p 1) in house dust mite sensitive patients with asthma or eczema, in comparison to non-allergic control patients who did not respond [5]. A significant positive correlation has also been shown between PBMC proliferative response and both allergy skin-test sensitivity and specific IgE production to ragweed antigens in highly allergic patients with rhinitis [6]. It has been suggested that sensitization to inhalant allergens can take place during fetal life as significant proliferative responses to both Der p 1 from the house dust mite and the major rye grass antigen Lol p 1 have been demonstrated at birth [7].

In this study we investigated the PBMC proliferative responses to inhalant and ingestant allergens at birth, 6 months and 1 year of age in babies with at least one allergic, asthmatic parent who were therefore at high risk of developing allergies themselves. The aim was to follow the course of the PBMC proliferative responses during the first year of life in relation to the subsequent development of allergy.

Materials and methods

Subjects

Patients attending antenatal clinics at the Princess Anne Maternity Hospital from January 1991 to February 1994, were approached and invited to enrol in a prospective study of the development of allergy in infants. Here we report on 88 babies of the 158 babies eventually enrolled, born to parents at least one of whom was allergic and asthmatic and 12 babies born to non-atopic parents and who completed follow-up to 1 year of age. These infants had PBMC proliferations performed at birth, 6 months and 1 year of age, skin tests and clinical assessments at 1 year of age and the atopic status of both parents assessed. The allergic, asthmatic status of the parents was established by clinical history, bronchial challenge and allergen skin-prick tests. Babies with one or both parents having a positive history, a 20% fall in lung function (PC₂₀) at a histamine concentration

of less than 8 mg/mL (using a modified Cockcroft technique [8]) and at least one positive skin-prick test to a common allergen were considered at high risk (HR) of developing allergies. Infants born to non-atopic parents (i.e. no allergic symptoms and no positive skin tests) were considered at low risk of developing allergies (LR).

Ethical approval was obtained from the Southampton Hospitals Joint Ethics Sub-Committee.

Samples

Umbilical cord blood was taken following normal deliveries of term babies from uncomplicated pregnancies. The blood samples were taken into heparin by needling from the placental end of the umbilical vein after cleaning with a swab (Steret, Seton Prebbles Ltd, Oldham, UK). Further venous blood samples were taken into heparin from the HR babies at 6 months and 1 year of age, however, for ethical reasons it was not possible to collect further samples from the LR babies.

Clinical assessment

The babies were clinically assessed at 6 months and 1 year of age by the same clinician (J.O.W.). Additionally, parents were encouraged to bring the infants for extra assessments if there was any persistent skin, bowel or chest problem. All evaluations were performed blind to the results of immunological tests. Asthma was suggested only if infants had more than either two separate wheezing episodes or two separate episodes of paroxysmal nocturnal cough with sleep disturbance for three consecutive nights or if deemed to require anti-asthma therapy. Eczema was diagnosed according to the criteria of Hanifin and Rajka [9] in infants with erythematous, papular, vesicular chronic skin eruptions with evidence of itchiness lasting for longer than 4 weeks and affecting either face (excluding nose and lips), torso and extensor surfaces of limbs or skin flexures.

Allergy skin-prick tests

The babies were skin-prick tested at 1 year of age to a positive histamine control (1%), negative control (phenol/saline 0.9%), grass pollen (2.5%), tree pollen (2.5%), cat dander (150%), dog dander (150%), house dust mite – *Dermatophagoides pteronyssinus* (1.2%), milk (50%), whole egg (6%) and wheat (10%) (SmithK-line Beecham, Welwyn Garden City, UK). It was deemed that at 1 year of age a weal size of ≥ 2 mm in the absence of a positive reaction to the negative control would be classified as a positive response.

This information was used to group the babies into

Table 1. Cord blood PBMC proliferative responses in counts per minute and allergic symptoms at 1 year of age in babies born to atopic parents who developed allergic symptoms by 1 year of age (i.e. HR group 1). ND indicates tests not done

	Proliferative responses (cpm)									Allergic symptoms	
	Unstimulated	α -CD3	PHA	HDM	Cat	Grass	Tree	BLG	OV	Eczema	Wheeze
1	283	4000	ND	2450	1969	4898	99	16825	24120	y	y
2	346	8750	ND	250	3364	1005	556	9420	4508	y	y
3	106	20793	ND	1700	471	1158	1933	19923	12133	y	y
4	1760	82841	ND	9927	3637	16479	8993	16261	16254	y	n
5	1581	68661	ND	834	1211	9929	11871	9422	17810	y	n
6	766	10625	ND	876	1921	3493	840	3971	1865	y	n
7	1249	3262	ND	7053	5833	7875	4506	7152	3837	y	n
8	2423	10670	ND	13843	16261	61636	10162	11158	16293	y	n
9	926	2585	ND	4782	14176	870	250	14070	7060	y	y
10	2095	1356	29837	5225	7146	2786	2932	12138	6213	n	y
11	450	5770	21659	5802	6029	1465	3099	11320	7878	y	n
12	1774	676	6747	1647	1417	330	122	2184	2079	y	n
13	2126	20861	3209	4786	3670	2595	115	4914	3723	y	n
14	1379	939	4991	1746	1119	1121	73	1735	1339	y	n
15	757	2832	10527	4963	7647	3731	3390	11528	6232	y	n
16	2350	5870	13338	2779	3520	2803	1032	4006	3269	y	n
17	455	822	10252	676	1167	1514	2824	2446	5594	y	n
18	5787	20221	43935	14152	7999	10868	15458	13747	8419	y	n
19	10228	28466	23267	9321	6562	11631	18014	12142	10868	y	y
20	5410	2185	16883	4049	4843	4069	5156	5633	5086	y	n
21	7329	3080	8468	7748	9294	12142	8660	8229	7615	y	y
22	1125	7033	31472	1461	2221	3656	3263	6428	3353	n	y
23	4858	3544	16512	4377	4421	7544	4261	5044	5131	y	y
24	2047	2585	6881	2025	2891	2167	2993	2658	2541	y	n
25	1486	11133	27554	1336	1359	452	995	1195	1653	y	n

y. Yes. n. No.

HR babies who had both allergic symptoms and positive skin-prick tests to one or more allergen (group 1, $n = 25$) (Table 1), HR babies who had no allergic symptoms or positive skin-prick tests by 1 year of age (group 2, $n = 21$), and HR babies with either allergic symptoms or positive skin-prick tests by 1 year of age (group 3, $n = 42$). As the allergic status of the 42 HR babies in group 3 could not be clearly established, they were not analysed further at this stage. A longer follow-up of these children is necessary. The 12 LR babies at 1 year of age were all skin-test negative and had no signs of allergic disease.

Proliferation assays

Peripheral blood mononuclear cells were separated from whole blood and cultured with or without stimulants as described previously [3]. Final concentrations of stimulants were as follows, PHA – 1 μ g/mL (Sigma, Poole, UK), anti-CD3 antibody – 50 ng/mL (Ortho Diagnostics, Raritan, NJ, USA), house dust mite – 2000 U/mL (National

Institute for Biological Standards and Control – First International Standard, Potters Bar, UK), cat fur extract – 2000 U/mL (NIBSC), timothy grass pollen (*Phleum pratense*) – 2000 U/mL (NIBSC), birch tree pollen (*Betula verrucosa*) – 2000 U/mL (NIBSC), betalactoglobulin – 10 μ g/mL (Sigma) or ovalbumin – 10 μ g/mL (Sigma). These concentrations were optimized as described previously [3]. Mitogenic responses to antigens were investigated by stimulating the PBMCs from five cord blood samples with the optimal doses of allergen for both 3 and 6 days. No positive proliferative responses ($\geq 2 \times$ background) were demonstrated in the 3 days cultures indicating that the allergens did not have a mitogenic effect.

Results were expressed in counts per minute as a ratio of incorporated [^{3} H] thymidine of stimulated cells to unstimulated cells.

Statistics

The Kruskal–Wallis test was used to analyse variance

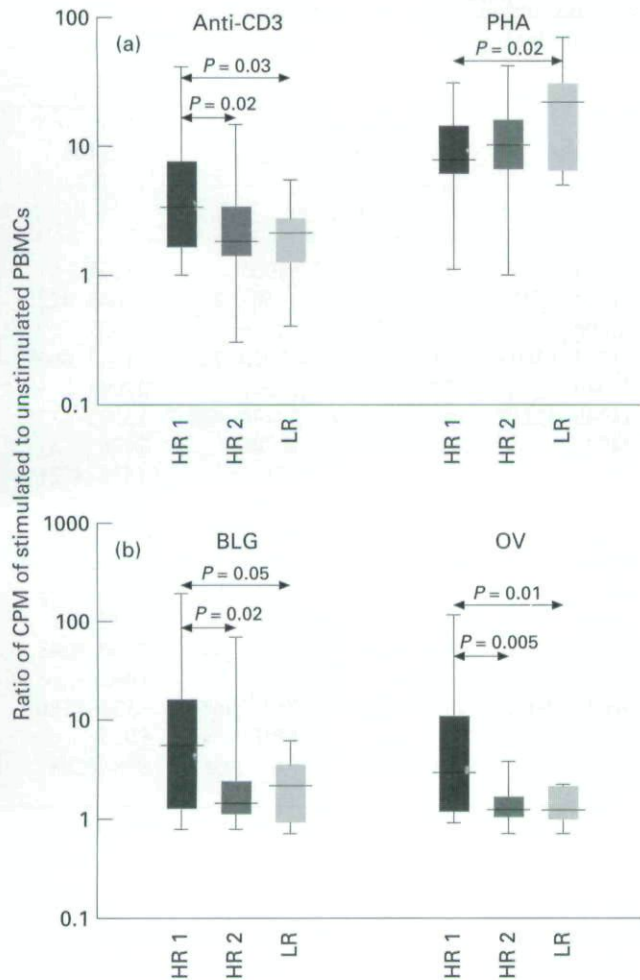


Fig. 1 (a) and (b) Peripheral blood mononuclear cell proliferative responses at birth in babies with a family history of atopy who developed allergic disease by 1 year of age (HR 1) or did not (HR 2) compared with babies with no family history of atopy (LR). Cells were stimulated with anti-CD3, PHA, betalactoglobulin or ovalbumin. The medians, interquartile ranges and maximum and minimum values are shown.

between HR groups 1 and 2 and the LR group. Where this test showed significant variation (anti-CD3, phytohaemagglutinin [PHA], betalactoglobulin and ovalbumin) the Mann-Whitney *U*-test was used to further analyse the data. The Wilcoxon rank sum non-parametric test was used to analyse differences between cord, 6 month and 1 year blood samples. Chi-square was used to compare infants with positive and negative proliferative responses with presence or absence of signs of atopy at 1 year of age.

Results

The atopic characteristics and individual proliferative

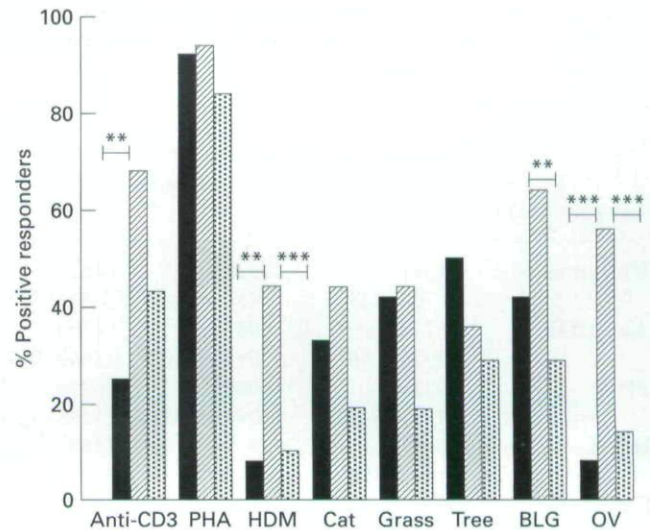


Fig. 2 Percentage of babies with a positive PBMC proliferative response at birth using a cut-off of a proliferation of ≥ 2 times greater than the unstimulated background proliferation and a value of ≥ 1000 counts per minute more than the background. Subjects were babies with a family history of atopy who developed allergic disease by 1 year of age (HR 1) or did not (HR 2) and babies with no family history of atopy (LR). Stimulants were anti-CD3, PHA, house dust mite, cat, grass pollen, betalactoglobulin and ovalbumin. (**= $P < 0.05$ and ***= $P < 0.01$). ■. HR2. ▨. HR1. ■. LR.

data of HR group 1 are shown in Table 1. These were 16 babies with eczema alone, two with asthma alone and seven babies with both eczema and asthma. All had a positive skin test response (≥ 2 mm weal) to one or more of the common allergens tested.

A significantly higher PBMC proliferative response to anti-CD3 antibody at birth was seen in the babies born to atopic parents at high risk of developing allergy who developed allergic symptoms by 1 year of age [HR 1] when compared with the babies born to atopic parents at high risk of developing allergy who did not develop allergic symptoms by 1 year of age [HR 2] and LR babies ($P = 0.02$ and $P = 0.03$). However, PHA-driven proliferative responses were significantly lower in the HR 1 babies than LR infants ($P = 0.02$) and showed a trend to be lower than the HR 2 babies (Fig. 1a) (Table 2).

Proliferative responses to betalactoglobulin and ovalbumin (OV) were higher in the HR group 1 babies than HR group 2 babies ($P = 0.02$ betalactoglobulin and $P = 0.005$ ovalbumin) or LR babies (betalactoglobulin $P = 0.05$ and ovalbumin $P = 0.01$) (Fig. 1b), (Table 2).

No significant differences in proliferative responses to anti-CD3, PHA or allergens were seen when comparing HR 2 babies with the LR babies at birth.

Table 2. Proliferative responses expressed as counts per minute showing medians and interquartile ranges at birth, 6 months and 1 year of age in babies born to atopic families who developed allergic symptoms by 1 year of age (HR 1) or did not (HR 2) and at birth in babies born to non-atopic families (LR)

Age (months)	HR 1			HR 2			LR
	0	6	12	0	6	12	0
Unstimulated	1581 762–2387	925 451–2241	1622 511–3235	2974 1710–6641	1590 1153–2518	1053 507–2183	3204 606–4225
Anti-CD3	5770 2956–15677	6083 2093–9750	4409 1469–9147	2606 1715–9760	1404 378–8411	2588 2020–7228	2625 1543–6669
PHA	14925 2728–26482	16099 11094–32351	26230 11698–42960	21940 11946–32257	24750 19581–43219	34735 24178–52207	27095 13592–43761
HDM	4049 1554–6458	639 264–2455	1580 951–4182	3472 1875–7278	1841 1123–2755	1074 521–2531	2476 1521–4721
Cat	3637 1669–6854	1119 568–4055	1883 620–3220	5080 1723–7631	1915 1094–3778	1753 881–2818	3760 1623–7082
Grass	3493 1312–8902	2491 654–2491	4409 4658–9695	4434 1628–7703	4211 1801–8079	4605 1028–10270	2471 2298–7473
Tree	2993 698–6908	1826 149–6217	4022 2006941	5626 1923–7266	3313 1937–4388	3292 2078–4916	6547 2500–9248
BLG	8229 39889–12945	1926 640–3060	1483 877–3609	5402 3602–10869	2024 1489–2871	1754 759–2967	4492 1836–8176
OV	5594 3311–9644	1610 724–2659	1630 755–4811	3498 2377–7339	1866 1318–3042	1701 543–2956	2925 938–6208

A positive proliferative response to a stimulant was taken as ≥ 2 times greater than the unstimulated background proliferation in addition to a proliferative response value of ≥ 1000 counts per minute above the

Table 3. Percentage of infants in HR1 ($n = 25$), HR2 ($n = 21$) at birth, 6 months and 1 year of age and LR ($n = 12$) at birth with proliferative response stimulation indices of ≥ 2 in addition to ≥ 1000 cpm above the unstimulated proliferative response

Age (months)	LR	HR 1			HR 2		
	0	0	6	12	0	6	12
α CD3	25	68	76	64	43	48	76
PHA	92	94	96	96	84	100	100
HDM	8	44	4	4	10	10	10
Cat	33	44	12	8	19	14	14
Grass	42	44	52	56	19	57	52
Tree	50	36	48	52	29	43	62
BLG	42	64	20	8	29	14	14
OV	8	56	12	4	14	5	14

background value was applied to the data (Table 3). Significantly more infants in HR 1 had positive proliferative responses to house dust mite at birth than in HR 2 ($P = 0.0097$) (Fig. 2). There were significantly more positive responders in HR 1 than HR 2 to betalactoglobulin ($P = 0.0166$) and ovalbumin ($P = 0.0035$). A strong trend for higher numbers of HR 1 babies with positive proliferative responses than HR 2 babies was also seen for anti-CD3, cat and grass.

When comparing HR 1 with LR infants significantly more babies in HR 1 had positive proliferative responses to anti-CD3 ($P = 0.014$), house dust mite ($P = 0.03$) and ovalbumin ($P = 0.0057$) (Fig. 2).

Comparisons of numbers of positive responders in the HR 2 and LR group showed no significant differences for any stimulant.

Longitudinally, proliferative responses to PHA increased significantly at 6 months ($P < 0.0001$ for both HR groups) (Fig. 3). A similar change was seen for the anti-CD3 with an increase in both HR groups at 6 months which was significant for HR 1 babies ($P = 0.0008$). Reactivity to house dust mite extract decreased at 6 months in HR group 1 ($P = 0.003$) (Fig. 4). Proliferations to cat fur extract fell at 6 months in all groups which was significant for the HR 1 babies ($P = 0.006$). Grass pollen

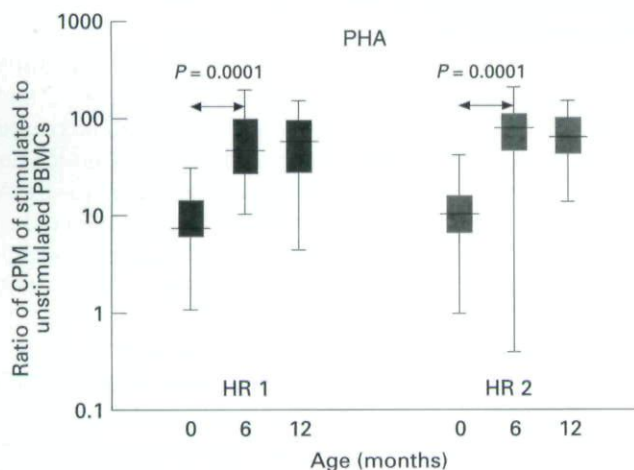


Fig. 3. Peripheral blood mononuclear cell proliferative responses at birth, 6 months and 1 year of age in babies with a family history of atopy who developed allergic disease by 1 year of age (HR 1) or did not (HR 2). Cells were stimulated with PHA. The medians, interquartile ranges and maximum and minimum values are shown.

reactivity increased at 6 months which was significant for the HR 2 babies ($P = 0.04$) (Fig. 5). An increase was seen in tree pollen reactivity in both groups at 6 months and again at 1 year. The increase seen from 6 months to 1 year was significant for the HR 2 infants ($P = 0.0009$). The proliferative responses to the food allergens betalactoglobulin and ovalbumin fell at 6 months in both HR groups.

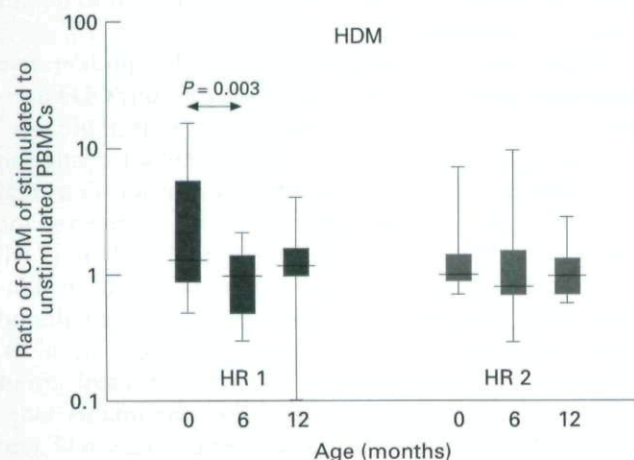


Fig. 4. Peripheral blood mononuclear cell proliferative responses at birth, 6 months and 1 year of age in babies with a family history of atopy who developed allergic disease by one year of age (HR 1) or did not (HR 2). Cells were stimulated with house dust mite extract. The medians, interquartile ranges and maximum and minimum values are shown.

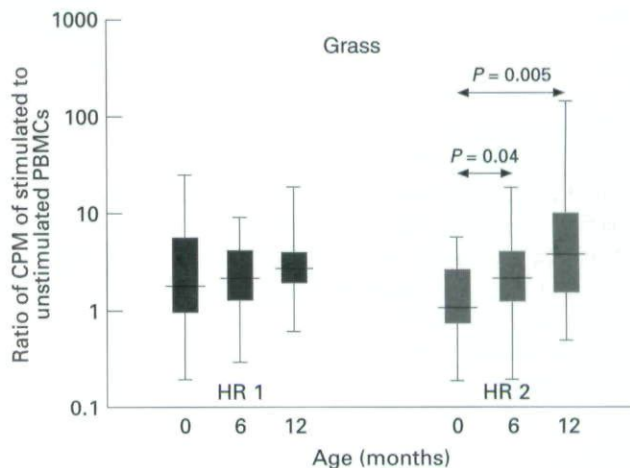


Fig. 5. Peripheral blood mononuclear cell proliferative responses at birth, 6 months and 1 year of age in babies with a family history of atopy who developed allergic disease by 1 year of age (HR 1) or did not (HR 2). Cells were stimulated with grass pollen extract. The medians, interquartile ranges and maximum and minimum values are shown.

The decrease over the first 6 months was significant in response to both betalactoglobulin (Fig. 6) and ovalbumin for HR 1 babies (betalactoglobulin $P = 0.0006$, ovalbumin $P = 0.004$, respectively).

At 6 months and 1 year no significant differences in proliferation ratios to anti-CD3, PHA or allergens were seen between the groups.

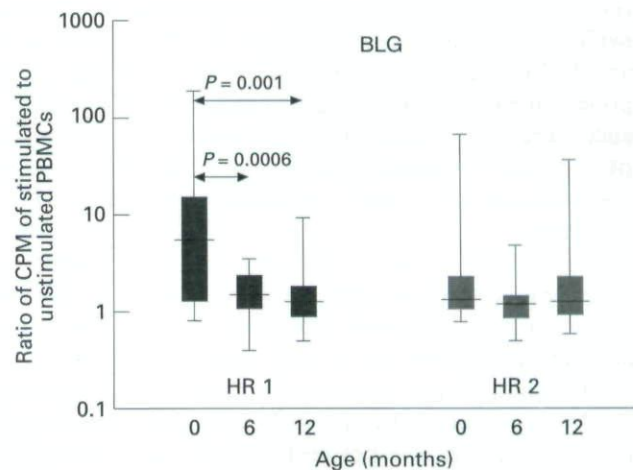


Fig. 6. Peripheral blood mononuclear cell proliferative responses at birth, 6 months and 1 year of age in babies with a family history of atopy who developed allergic disease by one year of age (HR 1) or did not (HR 2). Cells were stimulated with betalactoglobulin. The medians, interquartile ranges and maximum and minimum values are shown.

Discussion

The diagnosis of eczema and asthma at 1 year of age remains very subjective. It is for this reason that we have only accepted children with significant symptoms and signs classical of the two conditions and, in addition, evidence of atopy with positive skin-prick tests. For the diagnosis of eczema, we have employed the standard criteria as published by Hanifin and Rajka [9]. Furthermore, these assessments were done by the clinician blind to the results of the immunology and skin-prick tests. We believe therefore that there is no reason to doubt the diagnosis of eczema.

Prediction that an infant wheezer at 1 year of age will go on to have atopic asthma is currently not possible. The relationship between wheezing in infancy and later asthma, remains obscure. It is clear, however, that atopy is the most important factor in predicting the persistence and severity of wheezing in childhood [10–12]. As very few of the infants had wheezing and positive skin tests alone, they would not influence the results as presented. However, we believe that it is legitimate to include the wheezers with positive skin tests who, of course, also have a positive family history of atopic asthma. The probability that these children will have atopic asthma in later childhood is extremely high. Indeed, our own studies in such circumstances would suggest that the probability is close to 100% [13]. Further follow-up of these infants will be used to strengthen these observations.

Peripheral blood mononuclear cell proliferative reactions at birth were higher in infants born to atopic parents who developed atopic disease by 1 year of age (HR 1) when compared with the HR babies who had no evidence of atopy by 1 year of age (HR 2) in response to anti-CD3, extracts of house dust mite, cat fur, grass pollen, betalactoglobulin and ovalbumin. These differences were most pronounced for betalactoglobulin and ovalbumin. The presence of raised PBMC proliferative responses at birth imply intra-uterine sensitization to antigens.

Cord blood PBMCs from healthy babies with no detectable cord blood IgE have been shown to proliferate in response to Der p 1 (the major house dust mite allergen from *Dermatophagoides pteronyssinus*) and Lol p 1 (the major allergen from rye grass, *Lolium perenne*) [7]. More recently, Holt *et al.* demonstrated raised cord blood proliferative responses ($> 2 \times$ the proliferation of unstimulated cells) when stimulated by house dust mite allergen, rye grass allergen and ovalbumin [14]. They also suggested that this reflected allergen sensitization in fetal life. We believe that antenatal sensitization when it occurs increases the probability that early atopic disease will develop. Whether this is due to higher antenatal

allergen exposure or an innate difference in immune responsiveness remains to be established.

The proliferative response to PHA was significantly lower in the HR group 1 newborns than HR group 2 and LR infants at birth. By 6 months this had increased significantly in both HR groups and there was no longer a difference in the magnitude of response to PHA between HR 1 and HR 2 infants. This suggests that there is a transient maturational PBMC defect which takes longer to resolve in future atopics. The PHA triggered proliferation had further increased significantly in both HR groups, approaching adult levels, by one year of age. This transient lower PHA response by HR group 1 babies may suggest that either their lymphocytes were less mature at birth than the HR 2 babies or perhaps that these infants had a stronger suppressor response. The inability to respond as vigorously to the cross-linking of surface cell receptors by PHA might suggest a signal transduction failure such as implied by other studies of cord blood PBMCs [15,16]. A paradoxical strong suppressive effect of immature CD4⁺ cord blood T cells on T-helper function was demonstrated by Clement *et al.* [17]. A lower interferon-gamma production by cord blood PBMCs stimulated by PHA was seen in infants with a family history of atopy when compared with control groups. This reduced interferon-gamma production was not seen when cord blood PBMCs were stimulated with anti-CD3 [18]. Delayed maturation of infant T cells in high allergy risk infants was suggested as an explanation for reduced numbers of immunocompetent T cells from these infants when compared with low atopy risk infants [19]. Perhaps in these HR group 1 infants, cells are slower to mature than in HR babies who do not develop disease and in LR babies.

There was a general trend for the proliferative responses to non-specific stimuli such as anti-CD3 antibody and PHA to increase over the first year of life in all groups of infants. However, the pattern of change in responses to allergen fell into two categories. Perennial allergens such as house dust mite, cat fur extract and the two food allergens betalactoglobulin and ovalbumin fell over the first year in all groups. A reduction in ovalbumin stimulated PBMC responsiveness over childhood has been reported with peak reactivity seen at birth. However, this study looked at a random small group of babies at birth and did not follow the infants longitudinally [14]. The seasonal allergens, grass and tree pollen, showed the opposite pattern with higher proliferations at 6 months and 1 year than those seen at birth. The different patterns of change in antigen specific PBMC responses suggests that the nature of antigen exposure is important. Perennial antigen exposure results in reduced PBMC responsiveness such that they are less

reactive at 6 months and 1 year despite the increasing general reactivity to PHA stimulation. This might be due to the development of tolerance, such as suggested by Holt *et al.*, where ingestion of antigen can either result in T-cell anergy or the generation of antigen-specific suppressor T cells dependent on antigen dose [20]. However, the lowered PBMC responsiveness is most pronounced for the food allergens to which more than half of these infants develop positive skin tests by 1 year of age, thus tolerance may not be the most likely explanation. An alternative explanation is that sensitized lymphocytes move to the target organ where antigen exposure occurs such as the gut for ingestant antigens and the respiratory tract for inhalant antigens. Hamid *et al.* demonstrated higher numbers of activated T cells in the bronchial mucosa in severe asthmatics compared with mild asthmatics and non-atopic controls [21]. High frequencies of house dust mite specific T-cell clones have been reported in the skin of patients with eczema, demonstrating movement of antigen specific T cells to sites of inflammation. In this study greater numbers of house dust mite specific T-cell clones were found in the lesional skin than blood of these eczematous patients by an order of magnitude [22]. The presence of raised serum soluble IL-2R in early infancy, which is maintained above the adult range, in the absence of a concomitant increase in IL-2R bearing T cells in the blood suggests that the activated T cells were sequestered elsewhere, perhaps at mucosal tissue sites [23]. As the reduction in specific PBMC reactivity is more pronounced in the HR 1 babies this phenomenon may have a direct bearing on the later development of allergic disease. Further follow-up of these children is necessary to clarify this.

Response to seasonal allergens increased from birth through the first year. Holt *et al.* demonstrated increased PBMC response to stimulation to whole rye grass pollen extract from 6–10 weeks to 6 months of age. However, by 2 years of age the rye grass response had declined [14]. The increase seen in this study was only significant for HR 2 babies who did not develop atopic symptoms. This suggests that within HR group 1 some movement of peripheral T cells to target organs may be occurring, but not in HR group 2 where the infants are not sensitized. Perhaps a short high concentration exposure to allergen induces PBMC activation and proliferation but the exposure is not of a nature (i.e. too short and/or sporadic) to draw primed cells into target organs from the peripheral blood. It may take several years of short duration exposure for this to occur. This phenomenon might explain the relatively later onset of seasonal compared with perennial atopic disease in childhood.

The decrease in systemic PBMC response seen in both HR groups irrespective of the subsequent allergic disease

status due possibly to the loss of antigen specific T cells from the peripheral blood, recruited to sites of allergic inflammation suggests that allergen specific proliferative responses by PBMCs may not be a particularly useful tool for screening for allergy after environmental exposure of the baby to allergens. Cord blood proliferations may be the exception to this as they reflect systemic sensitization *in utero*, prior to the development of target organ inflammation.

Acknowledgements

We thank the National Asthma Campaign, British Lung Foundation, National Eczema Society and Barclay Foundation for supporting this work. We are also very grateful to the Child Health venesectioners and all the staff at the Princess Anne Maternity Hospital who have made this work possible.

References

- 1 Kondo N, Agata H, Fukutomi O, Motoyoshi F, Orii T. Lymphocyte responses to food antigens in patients with atopic dermatitis who are sensitive to foods. *J Allergy Clin Immunol* 1990; 86:253–60.
- 2 Kondo N, Fukutomi O, Agata H *et al.* The role of T lymphocytes in patients with food-sensitive atopic dermatitis. *J Allergy Clin Immunol* 1993; 91:658–68.
- 3 Warner JA, Miles EA, Jones AC *et al.* Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24:423–30.
- 4 Kondo N, Kobayashi Y, Shinoda S *et al.* Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992; 67:1003–7.
- 5 Rawle FC, Mitchell EB, Platts-Mills TAE. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P₁: comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 1984; 133:195–201.
- 6 Black PL, Marsh DG. Correlation between lymphocyte responses and immediate hypersensitivity to purified allergens. *J Allergy Clin Immunol* 1980; 66:394–401.
- 7 Piccinni M-P, Mecacci F, Sampognaro S *et al.* Aeroallergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993; 102:301–3.
- 8 Reiser J, Wadsworth J, Nunn AJ, Warner JO. Expression of results of histamine challenge. *Pediatr Pulmonol* 1989; 6:242–5.
- 9 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; 92 (Suppl): 44–7.
- 10 Van Asperen PP, Mukhi A. Role of atopy in the natural history of wheeze and bronchial hyperresponsiveness in childhood. *Pediatr Allergy Immunol* 1994; 5:178–83.
- 11 Clough JB, Williams JD, Holgate ST. The natural history of

- symptoms, peak expiratory flow, and bronchial responsiveness in 7 and 8-year-old children with cough and wheeze: a 12-month longitudinal study. *Amer Rev Respir Dis* 1991; 143:755–60.
- 12 Sporik R, Holgate ST, Cogswell JJ. Natural history of asthma in childhood – a birth cohort study. *Arch Dis Child* 1991;66:1050–3.
 - 13 Clough JB, Keeping KA, Warner JA, Warner JO. What factors predict persistence of wheeze in infants? Abstract, European Respiratory Society Conference, Barcelona, 1995 *Eur Respir J*, in press.
 - 14 Holt PG, O’Keefe P, Holt BJ et al. T-cell priming against environmental allergens in human neonates: sequential deletion of food antigen reactivity during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995; 6:85–90.
 - 15 Gerli R, Bertotto A, Spinozzi F et al. Phenotypic dissection of cord blood immunoregulatory T-cell subsets by using a two-color immunofluorescence study. *Clin Immunol Immunopathol* 1986; 40:429–35.
 - 16 Bertotto A, Gerli R, Lanfranccone L et al. Activation of cord blood T lymphocytes. *Cell Immunol* 1990; 127:247–59.
 - 17 Clement LT, Vink PE, Bradley GE. Novel immunoregulatory functions of phenotypically distinct subpopulations of CD4⁺ cells in the neonate. *J Immunol* 1990; 145:102–8.
 - 18 Rinas U, Horneff G, Wahn V. Interferon- γ production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy and Immunol* 1993; 4:60–4.
 - 19 Holt PG, Clough JB, Holt BJ et al. Genetic ‘risk’ for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992; 22:1093–9.
 - 20 Holt PG. Environmental factors and primary T-cell sensitisation to inhalant allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr Allergy and Immunol* 1995; 6:1–10.
 - 21 Hamid Q, Azzawi M, Ying S et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991; 87:1541–6.
 - 22 Sager N, Feldmann A, Schilling G, Kreitsch P, Neumann C. House dust mite-specific T cells in the skin of subjects with atopic dermatitis: Frequency and lymphokine profile in the allergen patch test. *J Allergy Clin Immunol* 1992; 89:801–10.
 - 23 Holt PG. Postnatal maturation of immune competence during infancy and childhood. *Pediatr Allergy Immunol* 1995; 6:59–70.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.