

Prenatal allergen contact with milk proteins

Z. SZÉPFALUSI, I. NENTWICH, M. GERSTMAYR, E. JOST, L. TODORAN,
R. GRATZL* K. HERKNER† and R. URBANEK

Department of Pediatrics and *Department of Neonatology, University of Vienna, and †Ludwig Boltzmann Institut, Vienna, Austria

Summary

Background Cellular proliferation to various allergens (*Dermatophagoides pteronyssinus*, β -lactoglobulin, bovine serum albumin, ovalbumin) has been found in cord blood cells. Whether this reflects a sensitization during foetal life is uncertain.

Objective We studied the cellular reactivity and cytokine production of cord blood cells in response to cow's milk proteins in a randomly selected group of newborns. The delineation of possible *in utero* allergen contact was attempted.

Methods Cord blood mononuclear cells from 39 neonates were incubated with cow's milk proteins (α -lactalbumin, β -lactoglobulin, casein, α -casein, β -casein, κ -casein, bovine serum albumin) for 7 days, and proliferation was assessed by incorporation of [³H]thymidine. Cord blood cell-derived interferon- γ (IFN γ) and interleukin-4 (IL-4) secretion was evaluated in response to allergen or phytohaemagglutinin (PHA) stimulation.

Results A pronounced proliferation of cells stimulated with α -lactalbumin (ALA; mean stimulation index 8.0, 95% confidence interval 5.2–10.8), β -lactoglobulin (BLG; mean stimulation index 5.9, 95% confidence interval 3.2–8.6) and α -casein (2.6, 95% confidence interval 2.9–9.1), as opposed to unstimulated cells in medium, was found. No correlation was found between cellular proliferation to milk proteins and parental atopy, maternal total IgE or cord blood IgE. IFN γ production (but not IL-4) was inducible by PHA (range 429–1810 pg/ml), but only in one individual upon stimulation with BLG. Preferentially, reduced IFN γ levels were found in individuals with positive parental allergic history.

Conclusion The recognition of allergen by cord blood cells indicates that allergen priming must occur prenatally. The relevance for subsequent sensitization is unclear.

Keywords: proliferative response, sensitization, cytokine, cord blood

Clinical and Experimental Allergy, Vol. 27, pp. 28–35. Submitted 21 July 1995; revised 25 April 1996; accepted 7 May 1996.

Introduction

The cellular basis for memory lies in the selective clonal expansion of antigen-specific T lymphocytes [1]. In this regard, elevated T-cell proliferation to an antigen is thought to represent a subsequent stimulation of previously primed antigen-reactive T cells [1]. The class of helper T cells (Th) involved in this interaction determines the type of immune response to a given antigen. Interleukin-4 (IL-4), secreted by the Th2 type of T cells, is involved in isotype switching

to IgE and therefore is relevant in the pathogenesis of type I hypersensitivity, whereas interferon- γ (IFN γ) is predominantly secreted by the Th1 type of T cells [2–5].

The functional maturity of neonatal cells is a point of controversy. It has been shown that T cells, isolated from foetal thymus tissue, can proliferate in response to phytohaemagglutinin (PHA) at 10 weeks of gestation [6]. Naive T cells appear immunocompetent in relation to IL-2 production, as well as proliferation to activation signals [7], but their capacity to secrete bioactive cytokines such as IFN γ and IL-4 is markedly reduced compared to adults [8,9]. Recently, reduced IFN γ secretion in neonates was attributed to functionally immature macrophages [7],

Correspondence: Dr Z. Szépfalusi, Department of Pediatrics, University of Vienna-AKH, Währinger Gürtel 18-20, A-1090 Vienna, Austria.

but was also positively correlated with subsequent development of atopy or atopic eczema [10–13]. Thus, while reduced cytokine production (in particular IFN γ and IL-4) appears to reflect an intrinsic maturation stage in neonate T cells, an additional reduction of IFN γ production might be the crucial defect in atopy-prone individuals.

Similar controversy exists concerning the predictive value of cord blood IgE for allergy development [14–16]. The effect of a positive family history and the gender of the infant, points towards a genetic influence on the immune system of the foetus. On the other hand, the influence of the maternal but not paternal atopy on cord blood IgE values would not be compatible with genetic influence. Perhaps some maternal factor acting through the placenta may be involved [17,18].

Very recently, it has been observed that T cells from newborns proliferate in the presence of β -lactoglobulin (BLG) [19], bovine serum albumin (BSA) [20], the major allergen of *Dermatophagoides pteronyssinus* (Der p 1) and the rye grass allergen (Lol p 1) [21]. The proliferation of neonatal cells when stimulated with BSA was found to correlate strongly with the subsequent development of allergic disease [20]. The enhanced T-cell reactivity to β -lactoglobulin in cord blood cells could be attributed to 'high risk babies' for allergy development [19] or atopic eczema [11]. Similarly, Picinni *et al.* found a significant stimulation of T cells to Der p 1 and Lol p 1, whereas no or very low proliferation was found in the same cord blood cells stimulated with tetanus toxoid antigen [21]. The immunological relevance of these observations is not clear. An *in utero* contact with the respective protein or peptide has been discussed [19,20].

In this study we tested the proliferative response of cord blood mononuclear cells from unselected individuals with a broad panel of milk proteins (α -lactalbumin, β -lactoglobulin, casein, α -casein, β -casein, κ -casein and bovine serum albumin). It was attempted to delineate possible *in utero* priming with antigens, in our case potential allergens. Furthermore, the postulated hypothesis of a predictive value of elevated cellular proliferative responses upon stimulation with allergens and a subsequent development of allergic diseases was discussed. The results were compared with the parental atopy history and total IgE levels in the blood of the newborn and the mother. IL-4 and IFN γ production was measured in response to PHA, α -lactalbumin (ALA) and BLG.

Materials and methods

Blood donors

Human umbilical cord blood was drawn on heparin before expulsion of the placenta [9], and cells were prepared as

described below. Mothers were asked about parental allergy history and total IgE was measured in cord and maternal blood. Neonates were classified as atopy-prone when one or more first-degree relatives (mother, father, siblings) had a history of asthma, atopic eczema or allergic rhinitis. The questionnaire comprised additional information about (a) seasonal or perennial occurrence of symptoms; (b) history of symptoms after contact with animals; and (c) worsening of symptoms during night-time.

Antigens

ALA, BLG, casein (CAS), α -casein, β -casein, κ -casein and BSA (Sigma, Deisenhofen, Germany) were used at final concentrations of 25 μ g/mL. Sodium dodecyl sulphate (SDS)–polyacrylamide-gel electrophoresis (PAGE) was performed, as described elsewhere, to confirm purity of the commercially available antigens [22]. PHA was used at a previously defined optimal concentration of 10 μ g/mL. For cytokine measurement antigens were used at a final concentration of 25 μ g/mL in 24-well plates.

IgE measurements

Cord blood and maternal serum IgE concentrations were measured by a commercially available enzyme-linked immunoassay (ELISA) (Kallestad, Sanofi Diagnostics Pasteur, USA). The sensitivity of the assay was 0.5 IU/mL. For exclusion of possible maternal blood contamination, cord blood serum was analysed for total IgA and IgM.

Cell preparation and T-cell proliferation

Cord blood mononuclear cells (CBMC) were separated from heparinized blood by density gradient centrifugation, and cultured at a concentration of 10^5 cells/200 μ L in UCC medium supplemented with 1% human serum, 2 mmol/L glutamine and 50 mg/mL gentamycin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO $_2$ for 7 days in the presence or absence of various milk proteins (ALA, BLG, CAS, α -casein, β -casein, κ -casein, BSA) [23]. IL-2 at 20 IU/mL was used as the positive control, medium alone as the negative control. After 6 days of culture, [^{3} H]thymidine ([^{3} H]TdR) (Amersham Int., Amersham, UK; 0.5 mCi/well) was added and the incorporated radioactivity was measured by scintillation counting after 16 h. Proliferation was expressed as a stimulation index (SI) of a geometric mean of triplicates. To compare responses between individuals, the results were expressed as SI because background proliferation varied considerably [24].

$$SI = \frac{\text{c.p.m. in cultures stimulated with allergen}}{\text{c.p.m. in unstimulated cultures}}$$

Cell separation

T cells were purified by rosetting with neuraminidase-treated sheep red blood cells (SRBC) and by density gradient centrifugation. To prevent proliferation, cell populations added as accessory cells were irradiated (2000 rads). Separated cells were cultured together in various proportions (5–10% accessory cells) at an overall concentration of $10^6/\text{mL}$.

Lymphokine measurements

IFN γ secretion by CBMC was determined using a commercial ELISA kit (Medgenix, Fleurus, Belgium), sensitivity threshold 0.03 IU/mL corresponding to 1.5 pg/mL. IL-4 levels were measured using a sandwich ELISA technique (Quantikine; R&D Systems, Minneapolis, MN, USA), sensitivity threshold 20 pg/mL. Recombinant and natural cytokine standards were used as references. Cells (10^6 cells/mL), freshly isolated under the conditions described above, were cultured in a 24-well plate for 48 h in the presence of ALA (25 $\mu\text{g/mL}$), BLG (25 $\mu\text{g/mL}$), PHA (10 $\mu\text{g/mL}$) or unstimulated (medium alone). The supernatants obtained from 23 subjects were recovered and stored frozen until the determination was performed.

Statistical analysis

Pair-wise comparisons between groups were carried out using the Mann–Whitney non-parametric *U*-test statistic.

Results

Parental history and maternal IgE

Of 39 parents, six (15.3%) reported having allergic symptoms (allergic rhinitis, atopic dermatitis, asthma). Total IgE was elevated in the serum of seven mothers (17.9%). Detailed data on history and IgE are shown in Table 1. Total cord serum IgE levels were measured between <0.5 kU/L and 4 kU/L. Of 39 newborns, three exhibited cord blood IgE higher than 1 kU/L (numbers 10, 27, 30), with 4, 2, 4 kU/L total IgE, respectively. In one mother/cord blood pair, an association in elevated IgE (356/2 kU/L) was found. Considering the cut-off level for elevated cord blood IgE as >0.5 kU/L, as suggested previously [14,20], the number of correlations between raised cord and maternal IgE concentrations increased to six pairs. The two newborns with cord blood IgE levels of 4 kU/L corresponded to the mothers' IgE of 28 and 26 kU/L, and a negative parental history. None of the cord blood samples was found to display IgM and IgA levels comparable with adult concentrations, suggesting no maternal blood contamination.

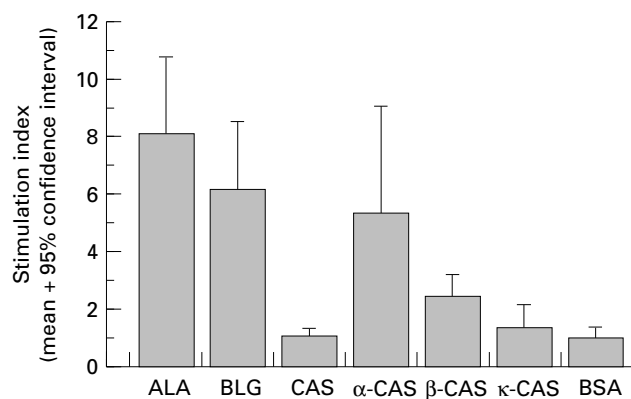


Fig. 1. Cord blood cellular proliferation to various milk proteins from unselected individuals. Cord blood samples ($n=39$) were incubated with 25 $\mu\text{g/mL}$ milk protein for 7 days and proliferation assessed by [^3H]TdR incorporation. Columns represent geometric mean of stimulation index; corresponding bars represent the 95% confidence intervals. Stimulation index is the ratio of stimulated cells with antigen and unstimulated cells in medium.

Proliferative responses of cord blood cells to milk proteins

Cord blood cells stimulated with milk proteins (ALA, BLG, CAS, α -casein, β -casein, κ -casein, BSA) exhibited proliferative responses in almost all subjects. The proliferation of CBMC to milk proteins was unrelated to the history or IgE level of the mother. Considering the stimulation index cut-off level of 2.0, 34 out of 39 newborns (87%) showed proliferative responses to ALA, 29 out of 39 (74%) to BLG, 7 out of 39 (17%) to CAS, 23 out of 39 (58%) to α -casein, 13 out of 39 (33%) to β -casein, nine out of 39 (23%) to κ -casein and four out of 39 (10%) to BSA. When the cut-off level was set at 3.0, the relation changed to 74, 59, 0, 49, 30, 13 and 7.5% positive responses, respectively. Cultures were routinely harvested at day 7 to ensure detection of any positive response. For the whole series, the maximal stimulation indices at 25 $\mu\text{g/mL}$ antigen are shown in Fig. 1.

The proliferative responses of CBMC to ALA, BLG and α -casein were by far more frequent (87%, 74% and 58%) and higher (SI, geometric mean, 8.0, 5.9 and 6.1, respectively) in comparison to the other proteins (SI, geometric mean, CAS 1.1, β -casein 2.4, κ -casein 1.6, BSA 1.1). In some of the samples, proliferation of cells to ALA and BLG was very high (patient numbers 5, 14, 15, 20, 27, 32, 35 and 36). Only in one patient (number 27), were high ALA, BLG and α -casein proliferative responses associated with elevated maternal IgE (356 kU/L), parental allergic history and elevated cord blood total IgE (2 kU/L). While elevated ALA and BLG stimulation levels were often found together, this was not the rule for elevated proliferative reactions to

Table 1. Patients' and parents' data

Subjects	Allergic history of the family	Mother's IgE (kU/L)	Cord blood IgE (kU/L)	Proliferative responses (c.p.m.) of CBL stimulated with							
				Medium	ALA	BLG	CAS	α -CAS	β -CAS	κ -CAS	BSA
1		837	<0.5	6040	36 194	24 488	3400	22 789	4831	5831	3332
2		27	<0.5	2787	156	7667	796	2785	2028	166	654
3		27	<0.5	8587	36 060	13 656	5361	20 666	7930	1621	3233
4	Positive	197	<0.5	6245	99 631	12 187	5478	6257	6741	6820	283
5		13	1	31 239	83 220	34 761	17 266	33 080	35 207	24 129	14 829
6		27	<0.5	10 286	22 726	22 125	9670	5143	16 113	111	18 619
7	Positive	273	1	5131	34 329	21 096	4917	27 318	6155	4932	4276
8		12	<0.5	8132	21 328	16 606	7477	14 574	7877	9818	2664
9		11	<0.5	1470	162	15 622	3950	22 204	13 291	9860	738
10		28	4	18 741	69 291	61 837	12 576	46 545	13 459	19 212	13 295
11		58	<0.5	4162	26 399	14 349	5485	13 529	7192	3162	2891
12	Positive	395	1	1470	162	15 622	3950	9361	13 291	1787	738
13		29	1	28 894	58 385	57 451	25 578	57 431	36 668	9141	24 041
14		42	<0.5	5127	17 537	12 067	329	229	3330	727	2232
15		66	<0.5	497	10 525	6226	360	330	783	202	571
16		75	<0.5	2387	17 991	30 524	5737	ND	10 299	ND	9741
17		26	<0.5	9441	52 519	32 259	13 636	32 691	28 336	7989	7601
18		35	<0.5	1777	309	2947	990	1481	2106	5078	1807
19		102	<0.5	1773	14 337	16 416	2971	9349	2408	1324	1436
20		70	<0.5	99	1991	161	218	3126	614	220	469
21		11	1	3519	16 909	6688	3264	7960	3570	2453	2453
22		85	<0.5	2438	23 574	20 134	1415	8790	4248	3524	1752
23		3	<0.5	5336	23 128	19 168	3828	ND	17 684	ND	8509
24		34	<0.5	1779	6818	5653	771	137	1201	1163	966
25		85	<0.5	1358	9251	11 272	2444	8557	4967	4126	1237
26		41	<0.5	1948	1670	8982	1192	222	1237	68	278
27	Positive	356	2	643	27 550	30 986	1407	19 253	5994	4261	1035
28		50	<0.5	4260	22 738	5684	3554	6216	4375	10 122	2842
29		3	<0.5	664	2923	246	563	3406	2279	1018	502
30		26	4	3469	28 106	16 853	2330	13 835	13 555	4301	3098
31		48	<0.5	6244	19 501	19 436	4339	18 485	5045	12 299	3517
32		52	<0.5	231	5257	4757	284	8511	356	1001	316
33		83	<0.5	3351	16 398	6587	1291	11 132	1887	1574	1229
34	Positive	>1000	1	547	3409	731	1119	883	343	255	507
35		73	<0.5	2366	32 861	27 360	291	238	370	242	4736
36		63	1	2706	47 938	12 848	1386	9453	3513	925	1076
37	Positive	200	<0.5	4017	9231	8831	1547	10 894	6422	582	1561
38		65	<0.5	6474	18 605	22 054	6470	ND	14 118	ND	7637
39		54	<0.5	6951	29 979	12 055	6245	123	3895	10 729	738

ND, not done.

Allergic history, mother's IgE level, cord blood IgE concentrations, proliferative responses of cord blood cells in response to milk proteins are shown in absolute values.

CAS, α -casein, β -casein, κ -casein or BSA. Therefore, an unspecific hyperreactivity of the neonatal cells was thought not to be the cause for the enhanced proliferative responses. Nevertheless, out of all casein proteins, α -casein was often

associated with elevated whey protein-induced cellular proliferation. This might account for allergenic potency of α -casein and whey proteins or preferential recognition by immature T cells.

Proliferative responses of separated T cells to milk proteins

To determine whether the elevated proliferative responses to milk proteins were of mitogenic nature, the proliferative effect on separated CBMC components, when stimulated with cow's milk proteins, was measured.

T cells separated by rosetting with SRBC showed excellent proliferation to milk proteins (ALA, BLG) when cultured with irradiated non-T cells. T cells alone did not proliferate in response to the antigens, presumably indicating a requirement for antigen-presenting cells and therefore excluding a mitogenic effect of the tested antigens (data not shown). As shown by previous investigators [24], non-T cells alone showed higher levels of background proliferation, but no specific response to milk proteins could be induced, ruling out a major endotoxin impurity of the commercially available extracts. These results indicate that the responding cells were separated by SRBC-rosetting in the T-cell population.

Cellular proliferative response in atopy-prone vs non-atopy-prone individuals

To examine the bias of atopy predisposition on proliferative responses of cord blood cells to milk proteins, cellular stimulation was compared between atopy-prone and non-atopy-prone individuals. The median values for ALA (6.45 in atopy-prone vs 4.4 in non-atopy-prone), BLG (3.15 vs 3.3), CAS (1.5 vs 0.7), α -CAS (4.0 vs 2.4), β -CAS (1.4 vs 1.27), κ -CAS (1.05 vs 0.8) and BSA (0.65 vs 0.7) were unexpectedly not significantly different (Fig. 2).

Lymphokine production to specific and unspecific stimuli

In order to attempt to discriminate between atopy-prone and non-atopy-prone individuals, CBMC were stimulated with milk proteins (ALA and BLG), PHA (10 μ g/mL) and medium control. IFN γ was not detectable under medium control incubation of the cells. Stimulation with PHA led to IFN γ production in almost all cord blood cell preparations, ranging from 429 to 1810 pg/mL. Under specific stimulation with milk proteins (ALA, BLG), IFN γ production was detectable in only one individual, in whom a positive association with parental allergy history was found (data not shown). As shown in Fig. 3, three out of 23 cord blood preparations had low IFN γ levels upon PHA stimulation. Of these three, two samples were confined to non-atopy-prone status, whereas the third preparation belonged to parents with positive atopy history. These differences were not significant. IL-4 was not detectable in any of the tested samples, reflecting a low level of IL-4 with values under the detection limit of the applied assay (data not shown).

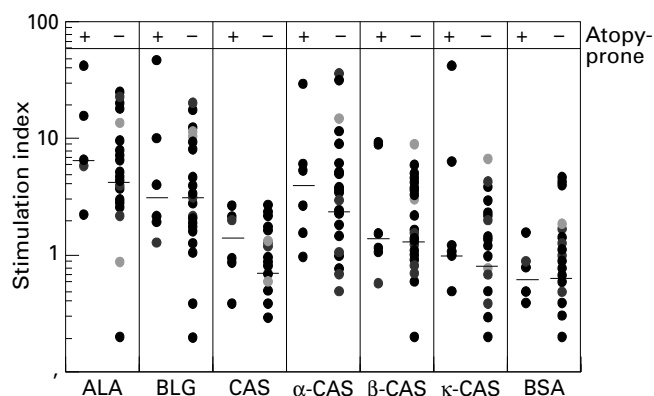


Fig. 2. Cord blood cellular proliferation to milk proteins in atopy-prone and non-atopy-prone individuals. Stimulation index from 33 preparations from newborns without parental allergic history were compared with six preparations from atopy-prone newborns. Median values are indicated in bars. Milk proteins are ALA, BLG, CAS, α -CAS, β -CAS, κ -CAS and BSA. Differences were not significant in any of the pairs, as determined by non-parametric U-test statistics.

Discussion

The present study examines the proliferative responses and cytokine production (IFN γ and IL-4) of cord blood cells from randomly selected, term newborns to cow's milk proteins.

Cow's milk proteins are usually the first allergens to which newborns are orally exposed after birth and after weaning. The first contact of the maturing newborn immune system with these potential allergens is thought to be crucial for the development or break-through of allergic diseases in 'atopy-prone' individuals [1–3]. The amount of potential antigen to which the gut is exposed during this time of life is

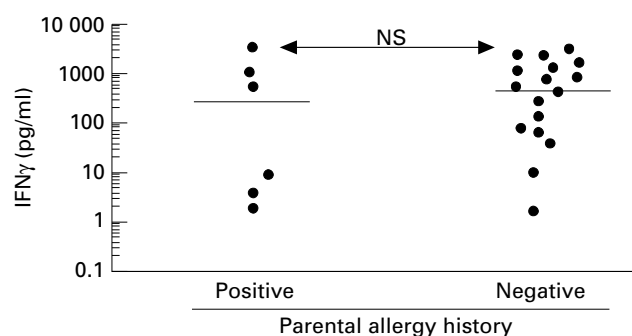


Fig. 3. IFN γ (pg/mL) production in response to PHA (10 μ g/mL) stimulation in cord blood cells from atopy-prone and non-atopy-prone individuals. 10^6 cells/mL were cultured with PHA (10 μ g/mL) and supernatant was harvested after 48 h. No significant difference was found between the two groups. Geometric mean values are represented by horizontal bars.

particularly important, because large amounts of antigen are thought to induce oral tolerance while smaller amounts might prime an immune response [25].

Although the elevated T-cell proliferative responses to α ALA, BLG and α -casein in a large majority of newborn cells was remarkable (Fig. 1), there was no correlation with the parental allergy history except in one case (number 27). Only six parents of investigated newborns reported suffering from allergic disease (asthma, atopic dermatitis, allergic rhinitis); seven mothers had elevated total IgE.

In contrast to previous studies, in which T cells were stimulated exclusively with BLG [19], the use of a broad milk protein sample allowed us to delineate preferential antigen recognition in neonates. In fact cord blood mononuclear cells reacted more strongly with ALA, BLG and α -casein (Fig. 1) than with other casein proteins and BSA. This might be due to preferential immunological recognition of whey proteins and some caseins by the maturing immune system, or to the biochemical properties of the antigens *per se*. We did not observe a predominant recognition of special milk proteins by 'atopy-prone' individuals, as neither parental history nor maternal total IgE and cord blood IgE correlated with measured proliferative responses (Table 1). Occasional elevation of proliferative responses of cord blood cells to antigen stimulation has been reported by other groups [11]; however, we noted a preferential recognition of antigens/allergens (ALA, BLG, α -casein). Warner *et al.* found high proliferative responses to BLG, cat fur and ovalbumin, raising the question of whether these observations might account for *in utero* priming [11]. In the case of perennial antigens (milk proteins, ovalbumin, cat antigen, house dust mite allergen), this mechanism might be valid, but the measurement of cellular proliferation in cord blood cells remains indirect evidence for *in utero* priming. In the case of seasonal allergens, an *in utero* contact of the foetal immune system with allergens depends on the time of exposure of the mother to the allergen. This is reflected by the finding of Picinni *et al.* [21], who found low and only occasionally proliferative responses to Lol p 1, the major allergen of ryegrass, in comparison to high and consistent responses to Der p 1, the major allergen of house dust mite. It might be assumed that the antigen or allergen exposure of the mother during pregnancy is responsible for possible prenatal allergen contact of the foetus. The exposure from the end of the first and during the complete second trimester could be of particular importance [9]. Using only perennial allergens in the form of milk proteins, we were unable to investigate this hypothesis in this study. Experiments with seasonal allergens (Bet v 1, Phl p 1; Van Duren *et al.*, *Pediatr. Res.*, in press), will give us more insight into the physiology of possible *in utero* priming.

In recent reports [13,19] enhanced BLG-triggered proliferation of cord blood cells was found in individuals with

parental allergic history. Using a similar technical design, our data do not support these findings. Warner *et al.* [11] also showed a positive correlation between a defective IFN γ production by cord blood mononuclear cells in response to anti-CD3 and BLG and an elevated proliferative response to BLG with subsequent development of atopic eczema at 1 year of age. No difference in anti-CD3-mediated IFN γ production in the atopy-prone and control groups was found by another group [12]. In our study, stimulation with PHA led to production of IFN γ in all samples (Fig. 3). In contrast, no IL-4 production was inducible or measurable with the applied technique, reflecting a T-cell mediated IFN γ response confined to the Th1 type of allergic stage. Moreover, reduced IFN γ levels in cord blood cells from 'atopy-prone' individuals were highlighted, suggesting a specific intrinsic maturation delay in this population (Fig. 3). With respect to the low number of newborns with positive family history, a definitive deduction is not valid from our data. This finding was accurate applying either positive parental allergic history or increased total maternal IgE as inclusion criteria for atopy-prone status. These findings are in agreement with previous studies [12].

On the basis of our results, we favour the hypothesis that the proliferation of newborn cells to various milk proteins does not differentiate between atopy-prone and non-atopy-prone individuals. On the other hand, as shown previously [10–12], reduced IFN γ production by polyclonal stimulation of total CBMC might be predictive for atopy development. From the six newborns with allergic history in the family, three cord blood cytokine stimulations revealed reduced IFN γ levels. Warner *et al.* [11] stressed the predictive value of BLG-specific responses at birth for the development of food-associated atopic dermatitis. However, the low IFN γ producer and control group overlapped over a wide spectrum. Thus the specificity and the sensitivity of IFN γ measurement for the prediction of allergy remains low [10,12]. Interestingly, in only one individual did BLG stimulation lead to measurable IFN γ levels, and here an association with positive allergy history in the family was evident. This is in contrast to previous findings [11,19], in which the proliferative response and IFN γ production upon BLG stimulation was measured exclusively. This discrepancy could be explained by the difference in study populations: unselected term populations in our study in contrast to atopy-prone individuals pre-selected by Warner *et al.* [11] and Piastra *et al.* [19]. However, this does not explain the discrepancy of mean proliferative responses to milk proteins found by us and recently by Little *et al.* [26]. Our data revealed high geometric mean levels of 8.0, 5.9 and 6.1 to ALA, BLG and α -casein, respectively, whereas Little *et al.* found mean values to whey and casein around 1.0. Different allergen sources might account for this, which confirms

the need for standardization of milk proteins for such *in vitro* testing.

In summary, our observations provide further evidence that allergen contact is already occurring prenatally. A promiscuous T-cell recognition of allergens in atopy-prone newborns is not favoured, because of unselected reactions towards ALA, BLG and α -casein in almost all newborns, unrelated to the atopy-prone status. Reduced IFN γ production in atopy-prone individuals might be relevant. The importance of *in utero* priming with antigens/allergens with respect to disease development remains to be answered.

Acknowledgements

This work was supported by BMWF 49.847, Fonds zur Förderung der wissenschaftlichen Forschung S06701-MED and Anton Dreher Gedächtnisstiftung 232/93. We thank collaborators for their help with this study: Dr Bartmann (Ulm, Germany) and Professor Husslein (Vienna, Austria).

References

- Bach JF. Definition of (immunologic) memory. In: JF Bach, ed. *Immunology*. New York: John Wiley & Sons, 1978:848–51.
- Lutzker S, Rothman P, Pollock R, Coffman R, Alt FW. Mitogen- and IL-4-regulated expression of germ-line Ig gamma 2b transcripts: evidence for directed heavy chain class switching. *Cell* 1988; 53:177–88.
- Bendelac A, Schwartz RH. Th0 cells in the thymus: the question of T-helper lineages. *Immunol Rev* 1991; 123:169–88.
- O'Hehir RE, Garman RD, Greenstein JL, Lamb JR. The specificity and regulation of T-cell responsiveness to allergens. *Annu Rev Immunol* 1991; 9:67–95.
- Wierenga EA, Snoek M, De Groot C *et al.* Evidence for compartmentalization of functional subsets of CD4⁺ lymphocytes in atopic patients. *J Immunol* 1990; 144:4651–6.
- Hayward AR. Development of lymphocyte responses and interactions in the human foetus and newborn. *Immunol Rev* 1981; 57:39–60.
- Ehlers S, Smith KA. Differentiation of T cell lymphokine gene expression: the *in vitro* acquisition of T cell memory. *J Exp Med* 1991; 173:25–36.
- Taylor S, Bryson Y. Impaired production of interferon- γ by newborn cells *in vitro* is due to a functionally immature macrophage. *J Immunol* 1985; 134:1493–7.
- Lewis DB, Yu CC, English BK, Kahn SJ, Wilson CB. Cellular and molecular mechanisms for reduced IL-4 and IFN γ production by neonatal cells. *J Clin Invest* 1991; 87:194–202.
- Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon- γ secretion in neonates and subsequent atopy. *Lancet* 1994; 344:983–5.
- 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; 24:423–30.
- 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 Immunol* 1993; 4:60–4.
- Miles EA, Jones AC, Warner JA, Warner JO. Lymphocyte proliferative responses to allergens and mitogens during the first year in babies born to atopic parents. *J Allergy Clin Immunol* 1995; 95:381 (abstr).
- Michel FB, Bousquet P, Greillier P. Comparison of cord blood immunoglobulin E and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol* 1986; 78:422–30.
- Delespesse G, Sarfati M, Lang G, Sehon AH. Prenatal and neonatal synthesis of IgE. *Monogr Allergy* 1983; 18:83–95.
- Host A, Husby S, Gjesing B, Larsen JN, Löwenstein H. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow milk allergy. *Allergy* 1992; 47:218–9.
- Magnussen CGM. Cord serum IgE in relation to family history and as a predictor of atopic disease in early infancy. *Allergy* 1988; 43:241–51.
- Arshad SH, Twiselton R, Smith J, Hide DW. Influence of genetic and environmental factors on the level of IgE at birth. *Pediatr Allergy Immunol* 1992; 3:79–83.
- Piastra M, Stabile A, Fioravanti G, Castagnola M, Pani G, Ria F. Cord blood mononuclear cell responsiveness to β -lactoglobulin: T-cell activity in 'atopy-prone' and 'non-atopy-prone' newborns. *Int Arch Allergy Immunol* 1994; 104:358–65.
- Kondo N, Kobayashi Y, Shinoda S *et al.* Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Chil* 1992; 67:1003–7.
- Picinni M-P, Mecacci F, Sampognaro S *et al.* Aeroallergen sensitization can occur during foetal life. *Int Arch Allergy Immunol* 1993; 102:301–3.
- Szépfalusi Z, Ebner C, Pandjaitan R *et al.* Alpha-livetin is a cross-reactive allergen in the bird-egg syndrome. *J Allergy Clin Immunol* 1994; 93:932–42.
- Ebner C, Szépfalusi Z, Ferreira F *et al.* Identification of multiple T cell epitopes on Bet v 1, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993; 150:1047–54.
- 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.
- Hanson DG. Ontogeny of orally induced tolerance to soluble proteins in mice: priming and tolerance in newborn. *J Immunol* 1981; 127:1518–24.
- Little SA, Bevin SV, Warner JO, Dean TP. Cord and six month

- blood lymphocyte responses to food antigens in breast and bottle fed babies. *J Allergy Clin Immunol* 1995; 95:331 (abstr).
- 27 Suomalainen H, Soppi E, Laine S, Isolauri E. Immunologic disturbances in cow's milk allergy. 2. Evidence for defective interferon- γ generation. *Pediatr Allergy Immunol* 1993; 4:203–7.
- 28 Hill DJ, Ball G, Hosking CS, Wood PR. Gamma-interferon production in cow milk allergy. *Allergy* 1993; 48:75–80.