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Cord blood B cells are mature in their capacity to switch to IgE-producing cells in response to interleukin-4 *in vitro*

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SUMMARY

Neonatal B cells have been considered immature because of their impaired capacity to produce immunoglobulins in response to polyclonal activators *in vitro*. Here we demonstrate that cord blood mononuclear cells (MNC) produce normal levels of IgE *in vitro* when cultured in the presence of interleukin-4 (IL-4), indicating that the B cells are mature in their capacity to switch to IgE-producing cells. However, in contrast to adult peripheral blood T cells, cord blood T cells failed to produce detectable levels of IL-4 upon activation by phytohaemagglutinin (PHA) concanavalin A (Con A) or combinations of PHA and the phorbol ester TPA. Interferon-gamma (IFN- γ) production by cord blood T cells following activation by Con A or PHA was also strongly reduced. However, high levels of IFN- γ , significantly higher than those produced by adult T cells, were synthesized in response to combinations of PHA and TPA, indicating that IFN- γ production by cord blood T cells is not intrinsically defective. In contrast, cord blood T cells produced levels of IL-2 that were significantly higher than those obtained by adult T cells tested in parallel. Collectively, our data indicate that the minimal levels of IgE production measured in cord blood (< 1 U/ml) are not due to immaturity of the cord blood B cells, but may be associated with the failure of cord blood T cells to produce detectable levels of IL-4, which has been shown to be responsible for induction of IgE synthesis both *in vitro* and *in vivo*.

Keywords interleukin-4 IgE synthesis cord blood mononuclear cells

INTRODUCTION

The reduced capacity of neonates to deal with a variety of infectious agents has been attributed to the immaturity of the immune system (reviewed by Andersson *et al.*, 1981). Despite the presence of a normal number of B cells, including normal percentages of IgM positive B cells CD4 and CD8 T cells and monocytes, neonates have very low serum IgM levels, and marginal levels of serum IgA and IgE, whereas the relatively high levels of serum IgG are of maternal origin (Campbell *et al.*, 1974; Andersson *et al.*, 1981; Hayward & Kurnick, 1981).

The impaired capacity to produce IgM, and the absence of significant endogenous IgG, IgA or IgE synthesis has been attributed to immaturity of the cord blood B cells, since their activation by polyclonal activators, like pokeweed mitogen (PWM), lipopolysaccharide (LPS) or Epstein-Barr virus (EBV), generally resulted in moderate or reduced levels of IgM synthesis and no IgA, IgG or IgE production (Andersson *et al.*,

1981). Defective immunoglobulin production has also been attributed to immature helper T cell functions or suppressor T cell activity (Tosato *et al.*, 1980; Andersson *et al.*, 1981; Olding & Oldstone, 1974; Oldstone, Tishon & Moretta, 1977; Durandy, Fischer & Griscelli, 1979).

However, neonatal T cell response to mitogens and alloantigens is in the normal range (Campbell *et al.*, 1974; Alford, Cartwright & Sell, 1976; Granberg, Manninen & Torvanen, 1980) but their cytotoxic activity is generally impaired (Stites, Carr & Fudenberg, 1974; Rayfield, Brent & Rodeck, 1980). Natural killer (NK) cell activity and antibody-dependent cellular cytotoxicity (ADCC) are also reduced in neonates (Granberg *et al.*, 1980; Baley & Schacter, 1979). Monocyte-macrophage functions are generally normal in neonates and their capacity to phagocytose bacteria and to present antigen is comparable to that of healthy adults. However, their chemotactic responsiveness is reduced (Weston *et al.*, 1976). Furthermore, interleukin-2 (IL-2) and interferon-alpha (IFN- α) production by cord blood mononuclear cells (MNC) was in the normal range, whereas IFN-gamma (IFN- γ) production was reported to be defective (Taylor & Bryson, 1985; Wakasugi & Virelizier, 1985).

Murine and human studies have recently indicated that IL-4 induces IgE synthesis (Coffman *et al.*, 1988; Finkelman *et al.*,

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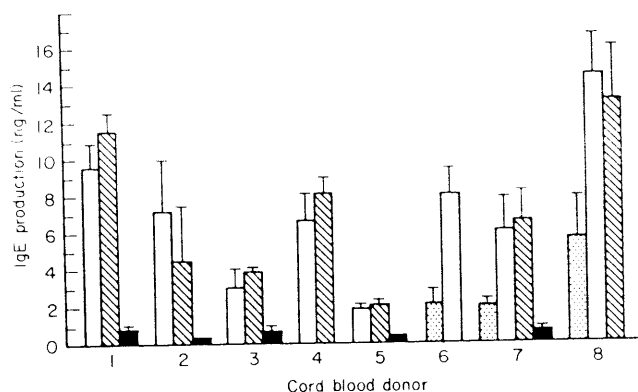


Fig. 1. Induction of IgE synthesis by cord blood mononuclear cells (MNC) by various concentrations of IL-4 (□, 100 U/ml; □, 500 U/ml; ■, 2000 U/ml; and ▨, 500 U/ml + anti-IL-4, 1:250) and its inhibition by anti-IL-4 antiserum. Pre-immune rabbit serum used as control was ineffective in inhibiting IL-4 induced IgE synthesis in cord blood samples 1-3, 5 and 7 (not shown). IgE production is expressed as mean $\pm$ s.d. of triplicate cultures.

1988; Pène *et al.*, 1988a, 1988b, Del Prête *et al.*, 1988) and evidence has been presented that IL-4 can act as an IgE switch-inducing factor (Rothman *et al.*, 1988; Lebman & Colfman, 1988).

In order to determine whether the virtual absence of IgE in cord blood was related to immature B or T cell functions, the capacity of cord blood MNC to produce IgE in response to exogenous IL-4 and their capacity to produce IL-4 upon activation was investigated. The present study demonstrates that cord blood MNC normally produce IgE in response to IL-4, but that these cells, in contrast to blood MNC of adult donors, fail to produce detectable levels of IL-4.

MATERIALS AND METHODS

Cytokines and reagents

Purified recombinant IL-4 (specific activity 1×10^7 U/ml) was kindly provided by Schering Research (Bloomfield, NJ). One unit of IL-4 is defined as the concentration of IL-4 inducing half-maximal proliferation of 5×10^3 T cells that were preactivated with PHA for 3 days. The monoclonal antibodies (MoAbs) OKT-3 (anti-CD3), OKT-4 (anti-CD4) and OKT-8 (anti-CD8) were purchased from Ortho Pharmaceutical Corporation (Raritan, NJ). The B cell-specific MoAb Leu-16 (anti-CD20) and the monocyte marker Leu-M3 (anti-CD14) were obtained from Becton Dickinson (Mountain View, CA). The polyclonal rabbit anti-IL-4 antiserum that blocks the biological activity of IL-4 has been described previously (Chrétien *et al.*, 1989).

IL-4-induced IgE synthesis

IgE synthesis induced by IL-4 was carried out in a two-step culture procedure, resulting in maximal IgE synthesis as described (Chrétien *et al.*, 1990). Cord blood MNC were collected from umbilical cord veins, upon delivery, and isolated by centrifugation over Ficoll Hypaque (Pharmacia, Uppsala, Sweden). The cord blood MNC or adult peripheral blood MNC (PBMC) were resuspended in Yssel's medium (Yssel *et al.*, 1984) supplemented with 5% fetal calf serum (FCS) and the cultures

Table 1. Effects of IL-4 on IgE, IgG, IgA and IgM synthesis by cord blood mononuclear cells

Cord blood donor	Incubated with	IgE (ng/ml)	IgG (ng/ml)	IgA (ng/ml)	IgM (ng/ml)
1	Medium	≤ 0.2	26 ± 2	8 ± 1	10 ± 3
	IL-4	9.5 ± 1.5	55 ± 4	9 ± 2	85 ± 6
	IL-4 + anti-IL-4	0.9 ± 0.2	19 ± 2	9 ± 1	12 ± 2
2	Medium	≤ 0.2	21 ± 1	6 ± 0	0 ± 0
	IL-4	7.1 ± 2.8	60 ± 4	7 ± 1	99 ± 8
	IL-4 + anti-IL-4	0.3	19 ± 1	6 ± 1	0 ± 0
3	Medium	≤ 0.2	27 ± 1	10 ± 2	23 ± 8
	IL-4	2.9 ± 1.1	63 ± 0	10 ± 0	140 ± 10
	IL-4 + anti-IL-4	0.5 ± 0.5	25 ± 2	9 ± 1	17 ± 2
5	Medium	≤ 0.2	22 ± 5	12 ± 4	0 ± 0
	IL-4	1.8 ± 0.2	19 ± 2	10 ± 1	0 ± 0
	IL-4 + anti-IL-4	≤ 0.2	24 ± 3	14 ± 2	0 ± 0
7	Medium	≤ 0.2	25 ± 2	16 ± 1	68 ± 3
	IL-4	6.1 ± 1.2	22 ± 4	12 ± 1	118 ± 7
	IL-4 + anti-IL-4	≤ 0.2	22 ± 2	15 ± 2	78 ± 9

IL-4 was added at 500 U/ml (50 ng/ml), anti-IL-4 at a dilution of 1/250. Pre-immune rabbit serum used as a control did not effect IL-4 induced IgE synthesis (not shown).

were carried out as described in the accompanying paper (Pastorelli *et al.*, 1990). Where indicated, anti-IL-4 antiserum was added at a final dilution of 1/500. Rabbit pre-immune serum was used as a control. The supernatants of these cultures were harvested and tested for their IgE, IgM, IgG and IgA content.

ELISAs for IgE, IgM, IgG and IgA

ELISAs for IgE, IgM, IgG and IgA were carried out as described in the accompanying paper (Pastorelli *et al.*, 1990).

Lymphokine production

PBMC or cord blood MNC were resuspended in Yssel's culture medium supplemented with 5% FCS at a concentration of 10^6 cells/ml. The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂ in 24-well plates (Becton Dickinson) in the presence of phytohaemagglutinin P (PHA) (Difco, Detroit, MI) at a final concentration of 100 µg/ml; PHA (100 µg/ml) in combination with the phorbol ester TPA (1 ng/ml) (Sigma); or Con A (10 µg/ml) (Pharmacia, Uppsala, Sweden). The supernatants of the cultures were harvested after 24, 48 or 72 h of incubation and stored at -20°C until testing for lymphokine activity.

Determination of IL-2, IL-4 and IFN-γ

The IL-2 activity of the supernatant was assessed by its ability to support the proliferation of an IL-2-dependent murine CTLL as described (Palard *et al.*, 1988; Pastorelli *et al.*, 1990).

IL-4 and IFN-γ present in the culture supernatants were quantified by immunoenzymatic assays performed as described previously (Chrétien *et al.*, 1989; Favre *et al.*, 1988). Sensitivity of the IL-4 assay was 50 pg/ml (0.5 U/ml), and the sensitivity of IFN-γ ELISA was 300 pg IFN-γ/ml (see Pastorelli *et al.*, 1990).

Table 2. Lymphokine production by cord blood and peripheral blood mononuclear cells following different modes of activation

Stimulus	Donor	Cord blood			Donor	Adult blood		
		IL-4 (pg/ml)	IL-2 (U/ml)	IFN- γ (ng/ml)		IL-4 (pg/ml)	IL-2 (U/ml)	IFN- γ (ng/ml)
Medium	1	<50	0.1	<0.3	1	<50	0	<0.3
PHA	1	<50	40.8	0.4 $\pm$ 0.0	1	94 $\pm$ 11	23	0.9 $\pm$ 0.1
PHA + TPA	1	<50	485.4	3.6 $\pm$ 0.0	1	278 $\pm$ 2	139	1.4 $\pm$ 0.1
Con A	1	<50	6.1	0.4 $\pm$ 0.0	1	77 $\pm$ 7	1	2.9 $\pm$ 0.4
Medium	2	<50	0	<0.3	2	<50	0	<0.3
PHA	2	<50	34.8	<0.3	2	240 $\pm$ 19	10	1.4 $\pm$ 0.1
PHA + TPA	2	<50	470.8	4.4 $\pm$ 0.0	2	358 $\pm$ 5	35	2.1 $\pm$ 0.2
Con A	2	<50	1.2	<0.3	2	59 $\pm$ 25	3	1.3 $\pm$ 0.1
Medium	3	<50	0	<0.3	3	<50	0	<0.3
PHA	3	<50	20.0	0.6 $\pm$ 0.0	3	189 $\pm$ 23	18	2.2 $\pm$ 0.1
PHA + TPA	3	<50	420.0	4.8 $\pm$ 0.1	3	474 $\pm$ 15	80	3.2 $\pm$ 0.2
Con A	3	<50	16.2	0.7 $\pm$ 0.1	3	71 $\pm$ 0	N.T.	1.6 $\pm$ 0.2
Medium	4	<50	0	<0.3	4	<50	0	<0.3
PHA	4	<50	49.2	0.5 $\pm$ 0.1	4	520 $\pm$ 21	38	2.9 $\pm$ 0.4
PHA + TPA	4	<50	367.6	5.5 $\pm$ 0.1	4	575 $\pm$ 41	50	3.6 $\pm$ 0.2
Con A	4	<50	28.2	0.6 $\pm$ 0.1	4	139 $\pm$ 2	4	1.5 $\pm$ 0.0
Medium	5	<50	0	<0.3	5	<50	0	<0.3
PHA	5	<50	29.8	0.7 $\pm$ 0.0	5	242 $\pm$ 21	20	0.8 $\pm$ 0.1
PHA + TPA	5	<50	189.5	4.1 $\pm$ 0.0	5	320 $\pm$ 31	124	1.2 $\pm$ 0.1
Con A	5	<50	22.4	0.4 $\pm$ 0.0	5	140 $\pm$ 2	8	1.3 $\pm$ 0.0
Medium	6	<50	0	<0.3	6	<50	0	<0.3
PHA	6	<50	38.0	0.5 $\pm$ 0.0	6	256 $\pm$ 21	24	1.2 $\pm$ 0.1
PHA + TPA	6	<50	158.3	6.3 $\pm$ 0.1	6	500 $\pm$ 15	96	2.0 $\pm$ 0.1
Con A	6	<50	13.5	<0.3	6	110 $\pm$ 4	5	1.5 $\pm$ 0.1
Medium	7	<50	N.T.	<0.3	7	<50	0	<0.3
PHA	7	<50	N.T.	0.8 $\pm$ 0.0	7	420 $\pm$ 9	15	0.9 $\pm$ 0.0
PHA + TPA	7	<50	N.T.	2.9 $\pm$ 0.0	7	620 $\pm$ 32	124	1.9 $\pm$ 0.1
Con A	7	<50	N.T.	0.6 $\pm$ 0.0	7	176 $\pm$ 8	7	1.4 $\pm$ 0.1

Mononuclear cells (1×10^6) were incubated in 1 ml volumes for 48 h. PHA was added at 100 μ g/ml, TPA at 1 ng/ml and Con A at 10 μ g/ml. NT, not tested.

Cell surface marker analysis

PBMC (10^6) were resuspended in PBS, in a volume of 100 μ l, and incubated for 30 min at 4°C with 5 μ l of anti-CD3, anti-CD4, anti-CD8, anti-CD20 or anti-CD14 MoAbs. Immunostaining and analysis were carried out as described in the accompanying paper (Pastorelli *et al.*, 1990).

Statistical analysis

Statistical analysis was carried out by Student's *t*-tests on paired or unpaired samples.

RESULTS

Induction of IgE synthesis by cord blood MNC by IL-4

As has been demonstrated by other investigators (Andersson *et al.*, 1981) the eight cord blood samples tested here contained normal percentages of CD3⁺, CD4⁺ and CD8⁺ T cells, B cells and monocytes as judged by staining with the MoAbs OKT-3, OKT-4 and OKT-8, Leu-16 and Leu-M-3 (not shown). In order to determine whether IL-4 could induce IgE synthesis by cord

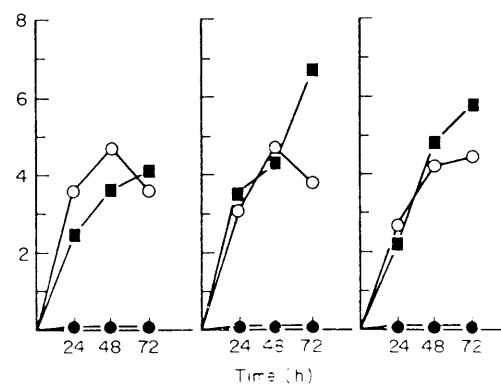


Fig. 2. Kinetics of IL-4 (●, pg/ml $\times 10^{-3}$), IL-2 (○, U/ml $\times 10^{-3}$) and IFN- γ (■, ng/ml) production by cord blood MNC from three donors following activation by PHA + TPA.

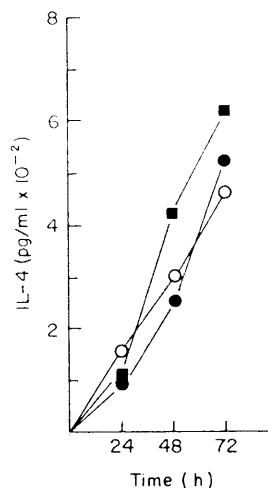


Fig. 3. Kinetics of IL-4 production by PBMC of three different adult donors following activation by PHA + TPA.

blood B cells, MNC derived from eight different cord blood samples were incubated in the presence of various concentrations of IL-4. In Fig. 1 it is shown that IL-4 at 100 U/ml (10 ng/ml) induced suboptimal IgE production (by MNC of donors 6–8). Maximal levels of IgE were generally synthesized in the presence of 500 U/ml of IL-4. IgE production was not further increased when the cells were incubated with IL-4 at 2000 U/ml. IgE synthesis was specifically induced by IL-4, since IgE production was completely blocked by anti-IL-4 antibody in the five cases tested (donors 1–3, 5 and 7; Fig. 1).

Effects of IL-4 on IgG, IgA or IgM synthesis

We have demonstrated previously that IL-4 predominantly induces IgE production, whereas the synthesis of the other isotypes was not affected, or, in the case of IgG, only marginally enhanced (Pène *et al.*, 1988b) and probably reflects the production of IgG4 (Lundgren *et al.*, 1989). Table 1 shows that 500 U/ml of IL-4 did not affect the low degree of spontaneous IgA production by the five cord blood MNC cultures tested, but IgG production was enhanced by two- to three-fold in three out of five donors (donors 1–3) tested. IgM production was enhanced three-fold or more in four out of five (donors 1–3 and 7) donors tested. IL-4 assayed at 500 or 2000 U/ml had comparable enhancing effects on IgG and IgM production (not shown). The enhancing effects of IL-4 on IgG or IgM production were completely inhibited by the anti-IL-4 antiserum (Table 1), confirming the notion that total IgG and IgM synthesis in these cultures are also either directly, as in the case of IgG4 (Lundgren *et al.*, 1989), or indirectly (for IgM) induced by IL-4.

Cord blood T cells fail to produce detectable levels of IL-4

Having established that cord blood B cells are able to produce normal levels of IgE in response to exogenous IL-4 *in vitro*, we investigated whether cord blood T cells were capable of producing IL-4. Table 2 shows that cord blood MNC from the seven donors tested failed to produce detectable levels of IL-4 after activation by Con A, PHA (100 µg/ml), or PHA (100 µg/ml) + TPA (1 ng/ml) for 48 h, whereas these cells produced IL-2 and IFN-γ, indicating that the lack of detectable IL-4 production was not due to insufficient activation of the cord blood MNC.

The overall levels of IFN-γ production after activation by PHA or Con A were significantly lower than those produced by PBMC isolated from adult healthy donors ($P < 0.01$ and $P < 0.001$, respectively), whereas levels of IL-2 production were significantly higher than those obtained by PBMC from adults tested in parallel ($P < 0.01$ and $P < 0.04$, respectively) (Table 2). Interestingly, the levels of IFN-γ and IL-2 production by the cord blood MNC after activation by the combination of PHA (100 µg/ml) + TPA (1 ng/ml) were much higher than those observed after activation by PHA or Con A. These IFN-γ and IL-2 levels were even significantly higher than those obtained by similarly activated PBMC from adults ($P < 0.01$ in both situations).

It could be excluded that kinetics of IL-4 production by cord blood MNC was different from that of healthy adult donors, since activation of cord blood MNC by PHA, Con A or PHA + TPA for periods of 24–72 h failed to result in detectable levels of IL-4 production, whereas maximal IL-2 and IFN-γ production was observed after 48 h and 72 h incubation, respectively, with the three donors tested (Fig. 2). In contrast, activation of PBMC of three adult donors resulted in significant levels of IL-4 production after 24 h, whereas maximal IL-4 production was observed after 72 h incubation (Fig. 3).

DISCUSSION

Cord blood B cells are considered to be immature because of their reduced capacity to produce immunoglobulins or to switch to IgG- or IgA-producing cells in response to polyclonal activators like PWM, LPS or EBV (a specific B cell stimulator). Generally the capacity to produce IgM in response to EBV or LPS is fully matured in neonatal B cells, but the cells fail to produce IgA or IgG (Andersson *et al.*, 1981). Activation of cord blood MNC by PWM resulted in a reduced production of IgM and no IgA or IgG synthesis (Andersson *et al.*, 1981). In addition to the immaturity of the B cells, defective isotype production has been attributed to immature helper T cell function (Andersson *et al.*, 1981) or suppression by autologous T cells (Olding & Oldstone, 1974; Oldstone *et al.*, 1977; Durandy *et al.*, 1979; Tosato *et al.*, 1980). Serum IgE levels are also strongly reduced in neonates, suggesting that neonatal B cells may also be immature in their capacity to differentiate and to switch to IgE-producing cells *in vivo*.

However, in the present study we demonstrate that cord blood MNC produce considerable amounts of IgE when incubated in the presence of IL-4. In the presence of saturating concentrations (500 U/ml) of IL-4, MNC of all eight cord bloods tested produced IgE levels varying between 1.8 and 14.1 ng/ml, the same range as the IgE production by adult PBMC (Pène *et al.*, 1988a, 1988b, 1988c; Del Prete *et al.*, 1988; Chrétien *et al.*, 1990). However, it should be noted that induction of optimal IgE synthesis by MNC from adult blood generally required slightly lower IL-4 concentrations (100–200 U/ml) (Pène *et al.*, 1988a, 1988b, 1988c). Induction of IgE synthesis was specifically mediated by IL-4, since it was completely blocked by an anti-IL-4 antiserum. These results clearly demonstrate that cord blood B cells are mature in their capacity to synthesize IgE at relatively high rates in the presence of IL-4, indicating that they can be induced to switch to IgE-producing cells by IL-4. IL-4 also induced some degree (two- to three-fold increase) of IgG synthesis by cord blood MNC of three out of

five donors tested, whereas IgM synthesis was considerably enhanced (three-fold or more) in four out of five cases tested. This induction of IgG and IgM synthesis was specifically mediated via IL-4, since the increase in IgG and IgM synthesis was completely blocked by the anti-IL-4 antiserum. The enhanced production of total IgG synthesis may reflect the induction of IgG4 synthesis, since it has been demonstrated that IL-4, in addition to inducing switching to IgE, can induce B cells to switch to IgG4-producing cells (Lundgren *et al.*, 1989).

The finding that IL-4 induces considerable levels of IgG and IgM production seems to be a particular property of cord blood MNC, since with blood or tonsil MNC from adults, only a very low degree of total IgG and no effect on IgM production was observed (Pène *et al.*, 1988b). The IgM-inducing effects of IL-4 are probably indirect and mediated via the release of other factors by the cord blood MNC in the culture, that enhance IgM synthesis following activation by IL-4.

We have demonstrated that although IL-4 is the sole inducer of IgE synthesis by MNC (Pène *et al.*, 1988a), it failed to induce IgE synthesis by highly purified B cells. Reconstitution experiments have indicated that in addition to IL-4, autologous CD4⁺ T cells are required for IgE synthesis and these IgE levels can be further enhanced by monocytes (Pène *et al.*, 1988b, 1989). The fact that cord blood B cells present in the MNC cultures (similarly as mature B cells) produce IgE in response to IL-4 indicates that the extremely low levels of serum IgE found in cord blood (cord blood serum IgE levels of the eight donors tested in this study varied between 0.8 and 1.7 ng/ml) and in very young children, are not due to immaturity of the B cells. Our finding that cord blood T cells fail to produce detectable levels of IL-4 (< 50 pg/ml; < 0.5 U/ml) upon activation *in vitro* suggests that the low levels of IgE in cord blood may be related to the absence, or to very low levels of IL-4 produced by cord blood T cells. It could be excluded that the failure to produce detectable IL-4 levels was due to suboptimal stimulation conditions, since activation of cord blood MNC with PHA, Con A, or a combination of PHA + TPA resulted in production of IL-2 and IFN- γ . In addition, aberrant kinetics of IL-4 production by cord blood T cells was ruled out.

Although the series of cord blood samples tested here is relatively small, IFN- γ production after activation by PHA or Con A was considerably reduced, compared with that by adult PBMC. In contrast, cord blood MNC produced quantities of IL-2 that were higher than those secreted by blood MNC of adult healthy donors, particularly after activation by PHA + TPA. Interestingly, this mode of activation also resulted in levels of IFN- γ production by the cord blood MNC that were higher than the quantities of IFN- γ secreted by blood MNC from adults activated in a similar way. Furthermore, Bryson, Winter & Gard (1980) reported that IFN- γ production by cord blood or newborn MNC upon activation by PHA was defective. However, other investigators have demonstrated that impaired IFN- γ production by cord blood MNC was not an intrinsic defect, but related to increased susceptibility of these cells to suppressive effects of PGE₂ (Wakasugi *et al.*, 1985).

Collectively, our results indicate that activation protocols resulting in optimal IL-2 and IFN- γ production fail to induce IL-4 production by cord blood MNC. Even upon activation by combinations of PHA + TPA which had been shown to be optimal for IL-4 production by adult PBMC and cloned T cells (Paliard *et al.*, 1988) failed to induce detectable levels of IL-4

synthesis. Our data furthermore indicate that different modes of activation of cord blood MNC result in quantitative differences in IFN- γ and IL-2 production. It remains to be determined why activation of cord blood MNC by PHA + TPA results in levels of IFN- γ and IL-2 production that are higher than those obtained by PBL of adults; however, it is clear from our data that cord blood T cells are not intrinsically defective in IFN- γ synthesis, but require particular activation signals. The finding that the cord blood MNC produced normal levels of IgE in response to exogenous IL-4, together with the fact that these cells produced non-detectable levels of IL-4 indicated that the minimal levels of IgE measured in cord blood and newborn blood are not due to the immaturity of the cord blood B cells, but may be related to lack of the cord blood T cells to produce detectable levels of IL-4 upon activation. Furthermore, these data suggest that the capacity of human T cells to produce normal levels of IL-4 may develop later in life, although it presently cannot be excluded that cord blood MNC, in contrast to adult blood MNC, upon activation produce factors suppressing IL-4 synthesis. Cell fractionation and T cell cloning experiments are currently in progress in order to determine more precisely the relative contribution of each of the cellular components in the cord blood MNC preparations to lymphokine production.

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