

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

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For newborn children both elevated serum IgE levels in the cord blood and a positive family history of atopic disease have been shown to be risk factors for the manifestation of atopic diseases. In adult patients with atopic dermatitis, *in vitro* interferon- γ (IFN- γ) production is reduced and a negative correlation with serum IgE levels has been shown. We have now raised the question if newborn infants at risk for the development of atopic disease have similar abnormalities of cytokine production at birth. *In vitro* production of interleukin 2, interleukin 6 and interferon- γ by peripheral blood mononuclear cells was measured in 53 newborns: 21 had cord blood IgE levels above 0.9 kU/l, 21 had a positive family history, 7 had both elevated IgE and a positive family history; 18 newborns with no identifiable risk for atopic disease served as controls. Umbilical cord blood mononuclear cells were stimulated with PHA or monoclonal antibody OKT3. *In vitro* production of interleukin 2 and 6 was comparable in all groups. Compared to controls IFN- γ production of peripheral mononuclear cells (PBMC) from newborns with elevated cord blood IgE was not different, but PBMC from newborns with a familial risk showed a significant decrease in PHA induced IFN- γ production ($p < 0.005$, U-test). No correlation between umbilical cord blood IgE and diminished IFN- γ production was found in newborns with or without a positive family history. We conclude that immunoregulatory abnormalities in newborns of atopic families are detectable already at birth and are unrelated to cord blood IgE.

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In atopic diseases multiple immunologic abnormalities have been described in the literature: Defective regulation of IgE-synthesis, decreased numbers of CD8+ T-cells and natural killer cells as well as decreased suppressor and killer cell function. In the murine system, IgE synthesis is controlled by two T-helper cell subsets, T_{H1} and T_{H2} , which exhibit different cytokine production profiles (1). Similar observations have recently been established in humans (2). T_{H1} -cells preferentially produce interleukin 2 (IL-2) and interferon- γ (IFN- γ). In contrast, T_{H2} are the main source of interleukin-4 (IL-4) and interleukin-5 (1). It is well known that human IgE synthesis *in vitro* is regulated by these T-cell factors (3). IL-4 induced IgE synthesis

by switching of unprimed B-cells to produce ϵ -chains and maintains IgE production by stimulation of B-cells (4). Conversely, IFN- γ is a potent inhibitor of both IgE synthesis and expression of the CD23 low affinity IgE-receptor (5).

In patients with atopic dermatitis *in vitro* production of IFN- γ has been shown to be profoundly decreased (6, 7). Serum IgE levels, *in vitro* spontaneous IgE production and expression of CD23 are elevated. Using these data some promising approaches using IFN- γ for treatment of atopic dermatitis have been made and a reduction of *in vitro* IgE production has been achieved during treatment (8, 9).

For newborns, a positive family history has been shown to be a risk factor for the development of atopic disease. A reduced IFN- γ production by neonatal T-cells in general compared to adults has been reported by several authors (10, 11). Unfortunately, none of these authors has taken a family history for atopy into account. As a second risk factor elevated IgE levels in the umbilical cord blood have been shown to be of value for prediction of atopic risk (12) as IgE does not pass the placental barrier. However, the relevance of elevated IgE levels in the umbilical cord blood to predict atopic disease has been questioned because a rapid decrease of IgE levels during the first week of life occurred in half of the infants (13).

No data are available addressing a possible correlation between elevated IgE and diminished IFN- γ synthesis in newborns. Therefore, we studied the production of cytokines involved in the regulation of IgE production by cord blood mononuclear cells. IL4 production, unfortunately, was mostly below the detection limits of our assay system and could, thus, not be analyzed. So, production of cytokines IL-2, IL-6 and IFN- γ in newborns with or without risk factors for atopic disease was investigated.

Material and methods

Patients and controls

Peripheral blood of 4 healthy adults (age 21-25 years) and umbilical cord blood of 105 consecutive term neonates was used for *in vitro* cytokine production. Atopic disease (bronchial asthma, allergic rhinoconjunctivitis, atopic eczema) in 1st degree relatives was found in 21 cases, elevated IgE levels (> 0.9 kU/l) in 21 cases (7 cases had both a positive family history and elevated IgE levels); 18 of the remaining 70 neonates were chosen as controls by random.

Culture conditions

Mononuclear cells were separated and washed from heparinized peripheral blood of healthy adult volunteers and umbilical cord blood according to methods described elsewhere (14). 1×10^6 cells/ml were cultured in a total volume of 1 ml in 48-well plates (Costar) in RPMI 1640 supplemented with antibiotics, L-glutamine, HEPES-buffer and 10% (v/v) fetal calf serum. Stimulation was performed with purified phytohaemagglutinin (PHA-P, Wellcome) and the T-cell stimulatory monoclonal anti-CD3 antibody OKT3 (Orthoclone). Unstimulated cultures were done in parallel.

In preliminary experiments, optimal stimulation conditions had been established using PHA at 1

$\mu\text{g/ml}$ and OKT3 at 5 ng/ml for IFN- γ and IL-2 production and PHA at 100 $\mu\text{g/ml}$ and OKT3 at 500 ng/ml for IL-6 production. Cultures were performed in duplicates at 37°C in a humidified atmosphere containing 5% CO₂. Supernatants were harvested after 24 h (IL-2-production) and 72 h (IL-6 and IFN- γ production). Supernatants were stored at -72°C. Determination of cytokine concentrations was done in batches after collection of supernatants of all 105 neonatal cord blood specimens.

Assays of cytokines and IgE

For determination of Interleukin-2 a radioimmunoassay (Medgenix) with a lower detection limit of 0.5 U/ml was used. Interleukin-6 was determined by ELISA (Medgenix, detection limit 3.5 pg/ml) and IFN- γ by radioimmunoassay (IRMA, Medgenix, detection limit 1 U/ml). All measurements have been performed in duplicates. The intra assay and inter assay variations were lower than 5% as stated by the manufacturer of the cytokine assays used. IgE was quantified by a commercial EIA (Phadezym IgE Prist Pharmacia, detection limit 0.35 kU/l).

Statistics

Statistical analyses were performed using Mann-Whitney-U-test, chi-square test and t-test.

Results

Cytokine production of newborn infants compared to adults

In preliminary studies, optimal concentrations of mitogens and kinetics of cytokine production were established. Conditions were found to be optimal for adults and newborns. Using these conditions, OKT3- or PHA-induced production of IL-6 was similar in newborn infants and adults. Maximum IL-6 production was reached after 72 h of culture and tended to be higher in neonates (kinetic data not shown). Because of small numbers of specimens investigated this difference lacks significance ($p = 0.08$, t-test). Maximum IL-2-production was also higher in the neonatal group. In newborns, peak production of IL-2 occurred after 24 h and decreased later on. Under the same conditions the peak of IL-2 production by adult lymphocytes was reached at 24-48 h. PHA or OKT3 induced IFN- γ production of both groups showed the same kinetics with a peak production reached after 72 h, but was significantly lower in newborns compared to adults ($p < 0.001$).

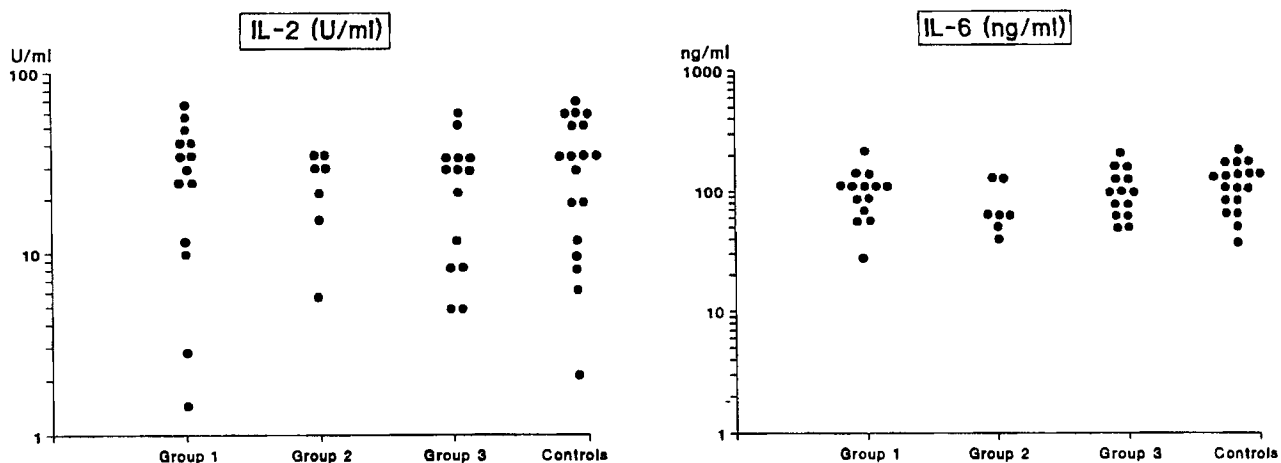


Fig. 1. IL-2 and IL-6 production of neonates at risk. PHA induced IL-2 (A) and IL-6 (B) production of PBMC from neonates with a positive family history (Group 1), neonates with both familial risk and elevated cord blood IgE (Group 2), neonates with elevated cord blood IgE alone (Group 3) and controls are shown. No differences were found between the groups and controls or between the different risk groups.

Cytokine production of newborns at risk

Of the 105 neonates there were 21 (20%) with a familial risk of atopic disease. Of these 7 (33%) showed elevated IgE of more than 0.9 kU/l in the umbilical cord blood. From the remaining 84 newborns 14 (17%) were selected according to their elevated IgE alone. This group was devoid of a positive family history. The frequency of elevated IgE in the group with a positive family history was not significantly different from the group without a family history (33% vs 17%, $p > 0.05$).

IL-2 and IL-6 production of newborn infants with a positive family history (Group 1), with both positive family history and elevated IgE levels (Group 2) in the umbilical cord blood, with elevated IgE levels alone (Group 3) and controls are

shown in Fig. 1. PHA-induced interleukin 2 production varied over a wide range. No significant differences were found between the groups. Using OKT3 as a T-cell mitogen, IL-2 production was detectable in only 4 of 14 cultures in Group 3, compared with 8 of 18 controls and 8 of 14 of Group 1. No IL-2 was detectable in the 7 cultures of Group 2. However, these proportions were not significantly different from each other ($p > 0.05$).

In our assay system, high amounts of IL-6 had been produced. IL-6 production after stimulation of cells with PHA was higher than OKT-3 induced IL-6 production in all groups. PHA induced IL-6 production tended to be lower in Group 3 than in controls. However, using the U-test no significant difference was found between the groups ($p = 0.07$).

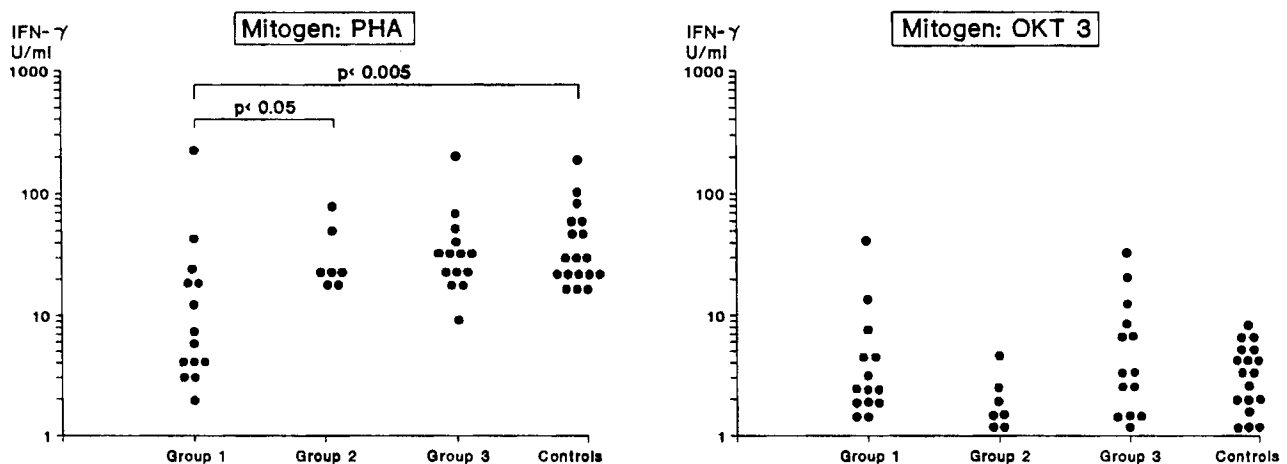


Fig. 2. IFN- γ production induced by PHA (A) and OKT3 (B). Neonates with a positive family history showed a significantly decreased IFN- γ production induced by PHA compared to controls ($p < 0.05$, Fig. 2A). This difference is exclusively due to diminished IFN- γ production in neonates with a positive family history, but normal cord blood IgE ($p < 0.005$). Neonates of Group 2 had a higher IFN- γ production compared with Group 1 ($p < 0.05$). OKT3-compared with PHA-induced IFN- γ production was lower in all groups ($p < 0.001$ for Group 1, Group 3 and controls and $p < 0.01$ for Group 2) and no differences were found between the groups (Fig. 2B).

Production of IFN- γ induced by OKT3 was markedly lower than PHA induced production in all groups (controls $p < 0.001$, Group 1 $p < 0.001$, Group 2 $p < 0.01$, Group 3 $p < 0.001$) as depicted in Fig. 2 A+B. No difference was found between PHA induced IFN- γ production in neonates with elevated IgE (Group 2 and Group 3) compared to controls. Using the Mann-Whitney U-test, PHA-induced IFN- γ -production in neonates with familial risk was significantly decreased compared to both controls and neonates with elevated IgE ($p < 0.05$). Especially in newborns with a positive family history but negative IgE (Group 1), IFN- γ production was significantly decreased compared to controls ($p < 0.005$).

In the 7 cases of Group 2 PHA induced IFN- γ production was comparable to IFN- γ production of the control group, but significantly higher than in neonates with a familial risk alone (U-test $p < 0.05$).

Discussion

The following major points emerged from the study:

- 1) PHA-induced IFN- γ production by cord blood mononuclear cells of neonates of atopic families is significantly reduced compared to age-matched controls. Therefore, immunoregulatory abnormalities in these newborns are already detectable at birth.
- 2) There is no evidence for a relationship between elevated IgE and diminished IFN- γ production in neonates.

IgE-production is up-regulated by several cytokines, most pronounced by IL-4. IL-2, IL-5 and IL-6 are further stimulatory factors (15–17). Conversely, IFN- γ is known to be a potent inhibitor of IgE production in adults (4, 18). The capacity of cord blood T-cells to produce IFN- γ is markedly decreased compared to adults (11). However, it was not known to date whether in the group of newborns displaying low IFN- γ production capacity there was a difference between those with or without a family history for atopy or those with or without an elevated cord blood IgE. Our study now indicates that in newborns with a family history for atopy IFN- γ production is significantly lower than in those without familial risk. In contrast, there is no relationship between IFN- γ production and cord blood IgE.

The reduced capability of cytokine secretion by neonatal lymphocytes compared to adult lymphocytes may be related to differences in the fre-

quency of naive versus memory CD4⁺ T cells (20, 22). In freshly isolated cord blood, most of CD4⁺ cells expressed the CD45RA phenotype of naive T-cells and only low numbers were of the CD45RO phenotype of memory T cells, expressing also some activation antigens like CD25, CD29 and HLA-DR antigens (21). While purified CD4⁺ CD45RO⁺ CD29⁺ cord blood T cells were equally effective in helper inducer function as the comparable adult cell population, their helper inducer function was profoundly suppressed even in the presence of small numbers of CD4⁺ CD45RA⁺ cord blood T-cells (22) which therefore have "suppressive" activity. This suppressor inducer function may also contribute to the reduced capability of neonatal T-cells to produce cytokines. However, *in vitro* activation of CD4⁺ CD45RO⁺ T-cells by PHA usually results in conversion to the CD45RA phenotype (22). In our experiments impaired production of IFN- γ by neonatal T-cells of newborns with positive atopic family history compared to the neonatal control population was also seen after stimulation of the cells with PHA and OKT3. However, PHA and OKT3 induced IL-2 and IL-6 production were comparable in all groups. Possible explanations for this are first an impairment of naive "suppressive" T-cells of newborns with atopic risk to become activated T-cells, second a prenatal acquisition of a specific suppressor activity toward IFN- γ production or an intrinsic inborn error of IFN- γ production, persisting also in the later life. IL-4 has been shown to be one possible inhibitor of IFN- γ production. Unfortunately, we were unable to detect IL-4 production by neonatal T-cells. A diminished *in vitro* response of neonatal T-cells to stimulation via the CD2 or the CD3 pathway by anti-CD3 antibodies is a well-known phenomenon (23, 24). This means that also in the individual neonatal T-cell the activation mechanism is controlled by so far unknown factors not present in the adult T-cells. A diminished capability to produce cytokines after stimulation with OKT3 was seen in all groups compared to PHA induced cytokine production and no significant differences were found between the groups.

Especially in neonates with a positive family history of atopic diseases, IgE levels were not correlated to decreased IFN- γ production. We found higher IFN- γ production in newborns with both elevated IgE and familial risk than in neonates with a familial risk only, which is in agreement with the observation that *in vitro* production of IgE by neonatal lymphocytes can be enhanced by IFN- γ (19). Production of cytokines exerting enhancing effects on IgE synthesis did neither differ significantly between adults and newborns nor among newborns of the different groups. IL-4, un-

fortunately, could not be measured because of insufficient sensitivity of the assay system.

Clinically, we do not yet know to date whether newborns with decreased IFN- γ production are more prone to develop atopic disease than those with age-related normal production. All children will have to be followed carefully in order to decide whether neonatal IFN- γ production has a predictive value with respect to the development of atopic disease within the risk group defined by a positive family history.

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