

Method of birth alters interferon-gamma and interleukin-12 production by cord blood mononuclear cells

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Many uncertainties exist regarding the capability of cord blood mononuclear cells (CBMC) to produce cytokines. A number of conflicting reports led us to examine the effects of method of birth on CBMC production of interferon-gamma (IFN- γ), interleukin-4 (IL-4) and interleukin-12 (IL-12). While constitutive production of IL-4 was found in both vaginally and cesarean-delivered infants, constitutive IFN- γ or IL-12 production was found in neither. CBMC from vaginally delivered infants responded to stimulation with concanavalin A/phorbol 12-myristate 13-acetate (Con A/PMA), phytohemagglutinin (PHA), and lipopolysaccharide (LPS) with significantly higher levels of IFN- γ than CBMC from unlabored cesarean section (CS) infants. Production of IL-12 was increased in the vaginally delivered group in response to LPS and PHA but not to ConA/PMA. In contrast, mode of delivery was not associated with differences in IL-4 production. These results indicate that mode of delivery significantly alters the capability of CBMC to produce some cytokines and therefore should be taken into account in interpreting fetal/neonatal mononuclear cell function studies.

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It has long been recognized that newborn and very young infants are at increased risk of serious infection. Though the reasons for this are not entirely clear, a number of differences in immunologic function between infants and adults have been described, including less efficient function of antigen-presenting cells (1), altered monocyte/lymphocyte cytokine production profiles (2–6), decreased natural killer (NK) cell function (7) and deficient neutrophil adhesion and migration (8). In addition, reductions in interferon-gamma (IFN- γ) production have been reported in infants at high risk of atopic disease as compared with infants not at high risk (9) or in infants who subsequently show increased prevalence of sensitization to asthma-associated allergens (10). We report dramatic differences in IFN- γ and IL-12 production associated with mode of delivery.

Materials and methods

Cord blood mononuclear cell isolation

Cord blood was obtained anonymously by umbilical venipuncture after delivery of the placenta. Blood was anticoagulated with sodium heparin and maintained at room temperature until processed. All samples were processed within 12 h of collection. Blood was layered over Ficoll®-sodium diatrizoate (density 1.077, LSM, ICN Biomedicals, Aurora, OH, USA) and centrifuged at 2000 *g* for 20 min at room temperature. The interface layer containing cord blood mononuclear cells (CBMC) and nucleated red blood cells (RBC) was carefully aspirated and mixed with dextran (Dextran 500, 6% in 0.9% NaCl, Sigma, St. Louis, MO, USA) and incubated at 37°C for 10 min. The mixture was then layered over Ficoll®-sodium diatrizoate and

centrifuged as above. The dextran sedimentation and density centrifugation steps were repeated up to three times as necessary until no RBC contamination was visible.

CBMC culture and stimulation

Cells were cultured at 10^6 cells/ml in RPMI-1640 (Gibco-BRL, Grand Island, NY, USA) with 10% autologous plasma, HEPES (1 M; Gibco-BRL), L-glutamine (200 mM; Gibco-BRL) and pen/strep (10,000 units/ml/10,000 µg/ml; Gibco-BRL) at 37°C in 5% CO₂. Cells were cultured under control conditions or were stimulated using 10 µg/ml concanavalin A and 10 ng/ml phorbol 12-myristate 13-acetate (ConA/PMA, Sigma, St. Louis, MO, USA), 2.5 µg/ml phytohemagglutinin (PHA, Sigma) or 10 µg/ml lipopolysaccharide from *Escherichia coli* (LPS, Sigma). Each condition was cultured in triplicate. Supernatants were harvested at 24, 48 and 72 h (from separate cultures) and stored at -70°C until assayed for cytokines.

Cytokine assays

CBMC supernatants were assayed for IFN-γ, interleukin-4 (IL-4), and interleukin-12 (IL-12) by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Limits of detection are 15.6, 0.25, and 7.8 pg/ml, respectively. Levels of soluble CD14 (sCD14) were measured by ELISA using a commercially available kit (R & D Systems) according to the manufacturer's instructions. Limit of detection was 250 pg/ml.

Statistics

If not normally distributed, data were log transformed to achieve normal distribution. Normally distributed data were analyzed by the Student's *t*-test. Non-normally distributed data were analyzed by the Mann-Whitney *U*-test. All analyses were performed using the Statistical Package for the Social Sciences (v7.5, SPSS Inc., Chicago, IL, USA).

Results

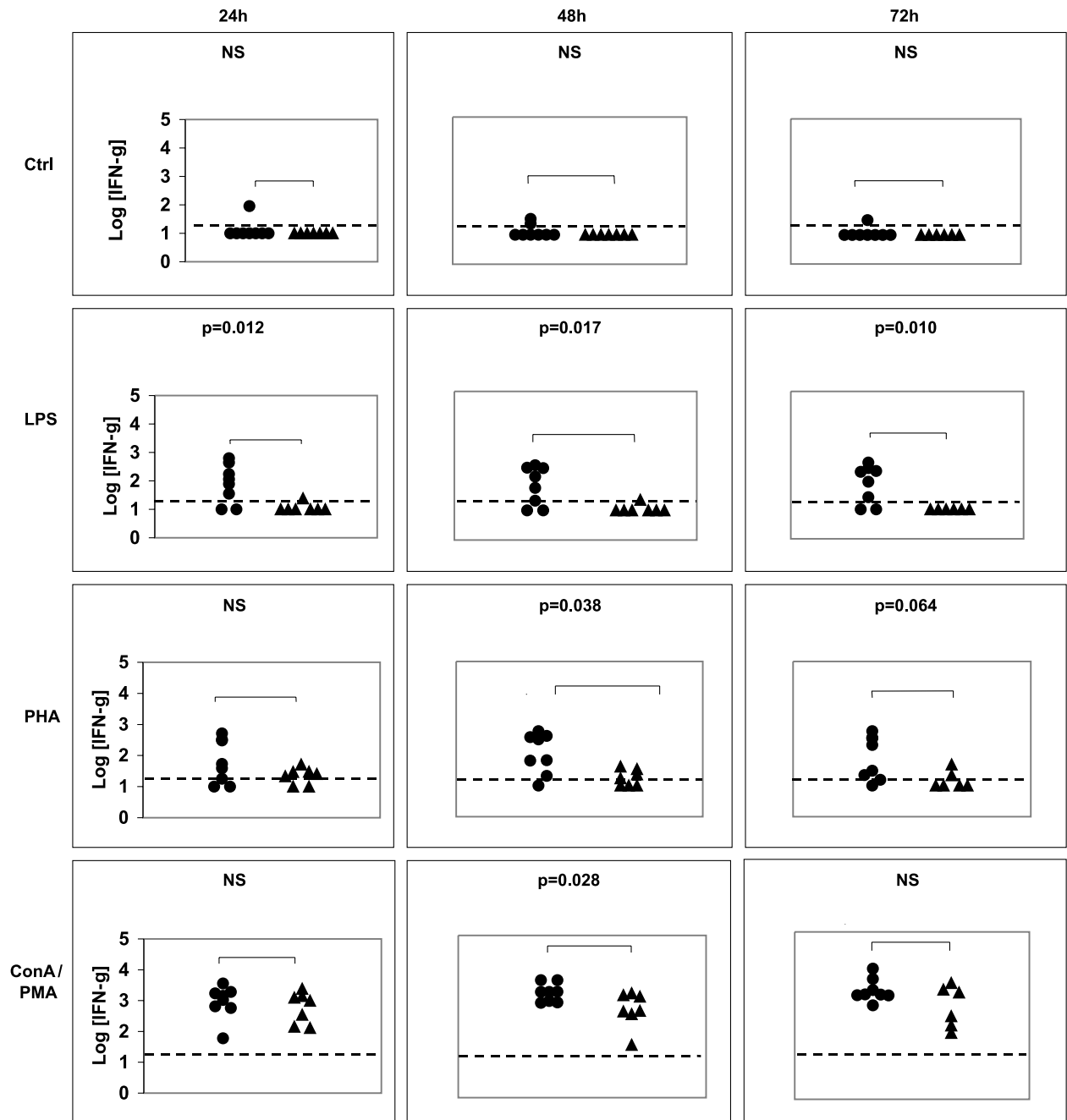
CBMC were isolated from 16 term newborns, 8 delivered by unlabored cesarean section (CS) and 8 vaginally. Constitutive IFN-γ production was largely undetectable in CBMC supernatants from both vaginal- and CS-delivered infants. Low levels of IFN-γ were detected in one sample at

24, 48 and 72 h and one sample at 48 h, both from vaginally delivered infants (Fig. 1). CBMC from infants delivered vaginally produced higher amounts of IFN-γ after stimulation at all three time points following LPS stimulation, and at 48 h following ConA/PMA and PHA. The difference was most pronounced 48 h after LPS stimulation. No differences were observed in IL-4 production either constitutively or in response to stimulation (Fig. 2). Constitutive IL-4 production was detected in 9 of 15 samples at 24 h (5 vaginal; 4 CS), 8 of 15 samples at 48 h (5 vaginal; 3 CS) and 8 of 14 samples at 72 h (6 vaginal; 2 CS). While IL-4 production increased significantly from constitutive levels in response to ConA/PMA and PHA, there was no increase after stimulation with LPS. Because of limited availability of supernatants, IL-12 was assayed at 24 and 72 h only. Constitutive production of IL-12 was not detected in either group (Fig. 3). IL-12 was produced after 24 h in response to LPS (4/6 vaginal; 1/5 CS), PHA (4/6 vaginal; 0/6 CS) and ConA/PMA (4/6 vaginal; 2/6 CS) with higher production in the vaginal group in response to PHA ($p = 0.022$). There was a trend toward higher production in the vaginal group with LPS ($p = 0.07$). LPS was the only stimulus associated with detectable IL-12 levels at 72 h. At this time point there was significantly higher IL-12 production in the vaginal group in response to LPS ($p = 0.009$). Because the greatest effect was noted in response to LPS, cord plasma levels of sCD14 were measured. sCD14 was detectable in all samples (median 0.7 µg/ml vaginal, 0.6 µg/ml CS), but there was no difference between groups (data not shown).

Discussion

As our results clearly demonstrate, mode of delivery has a significant influence on stimulated IFN-γ production by CBMC. While this effect was seen with a variety of stimulating conditions, it was most pronounced after stimulation with LPS. Response to stimulation reached maximal levels primarily in the first 24 h.

Labor has been reported to enhance NK cell activity in cord blood (11). Pittard and colleagues have also reported that labor results in decreased T cell proliferative capacity in response to mitogen, decreased B cell differentiation and decreased percentage of CD4+ cells in cord blood (12,13). Importantly, the phenotypic character of the lymphocyte populations was dependent upon the experience of labor and independent of the subsequent mode of delivery of the infant (14). It is unknown whether the labor-associated



decrease in CD4+ cells is evenly distributed among Th1 and Th2 cells. A selective decrease in Th2 lymphocytes as the result of labor or a decrease in Th1 cell number or function associated with CS delivery could potentially account for our findings.

The cellular source(s) of the IL-12 and IFN- γ measured are not discernible from our data. Within the CBMC population, IFN- γ could be produced by CD8 $^{+}$, Th1 CD4 $^{+}$ lymphocytes,

or NK cells. The source of IL-12 is more restricted with monocytes almost certainly the primary source. Circulating dendritic cells are a potential source of IL-12, though the numbers of these cells within the CBMC population are quite small in comparison to monocytes.

Our findings are consistent with those of Scott et al. (15), who reported *Staphylococcus aureus*-induced IFN- γ production by CBMC, which was associated with increased levels of

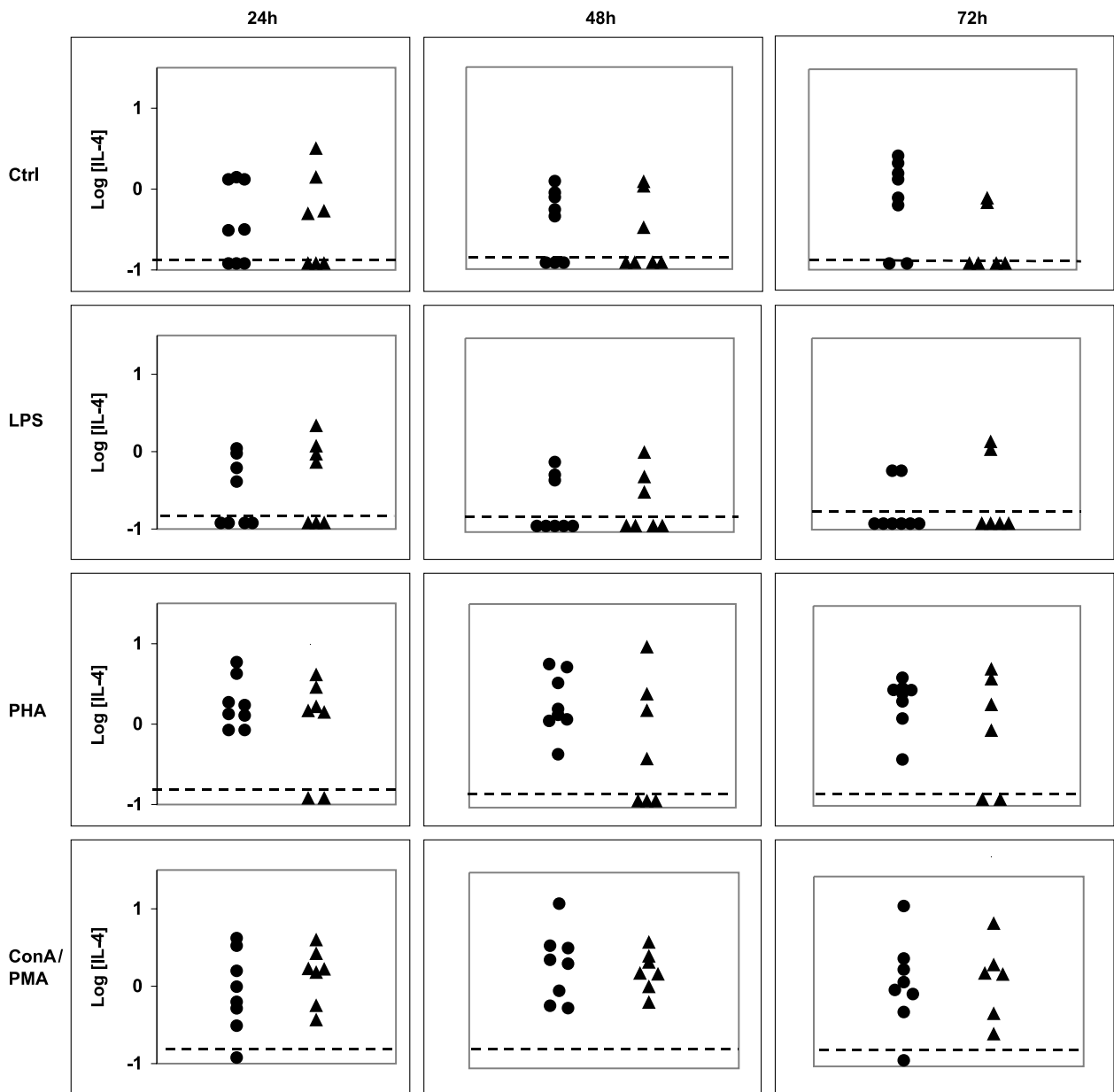


Fig. 2. IL-4 production in CBMC. Stimulation conditions were as follows: LPS = 10 $\mu\text{g/ml}/10^6$ cells; PHA = 2.5 $\text{mg/ml}/10^6$ cells; ConA = 10 $\text{mg/ml}/10^6$ cells; PMA = 10 $\text{ng/ml}/10^6$ cells. Circles and triangles correspond to vaginal delivery and cesarean section delivery samples, respectively. Dashed line represents the limit of detection. The difference between groups was not significant.

IL-12 as well. We found early increases in IL-12 in response to PHA and LPS among vaginally delivered infants. The response to LPS was still detectable at 72 h. Interestingly, the results of Scott et al. are in contrast to those of Lee et al. (16) using the same stimulus, but without induction of IFN- γ . These findings appear resolvable by our data as the CBMC in Lee's study were isolated from infants delivered by unlabored CS, while Scott's population was predominately vaginally born. These two studies further suggest that IL-12 may be influenced by labor as

well, and may be the mechanism for IFN- γ up-regulation because IL-12 has been shown to regulate IFN- γ production (17).

Combined with those discussed above, our findings suggest that vaginal delivery induces changes in the newborn immune system that may enhance the infant's ability to combat microbial infection immediately after birth. Indeed, the greatest differences were noted in CBMC stimulated by LPS, perhaps suggesting that this observation is due to increased IFN- γ production by monocytes or NK cells. If so,

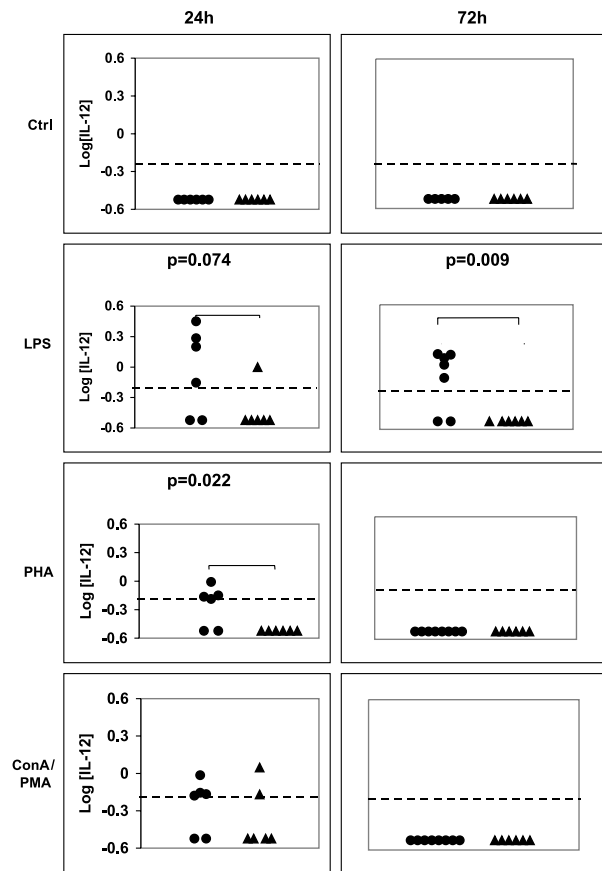


Fig. 3. IL-12 Production in CBMC. Stimulation conditions were as follows: LPS = 10 $\mu\text{g/ml}/10^6$ cells; PHA = 2.5 $\text{mg/ml}/10^6$ cells; ConA = 10 $\text{mg/ml}/10^6$ cells; PMA = 10 $\text{ng/ml}/10^6$ cells. Circles and triangles correspond to vaginal delivery and cesarean section delivery samples, respectively. Dashed line represents the limit of detection.

differences in CD14 expression may be a key factor. While we did not evaluate expression of membrane-bound CD14, we were unable to detect any differences in cord plasma sCD14 between the two groups.

The duration of this effect is unknown. The exact mechanism by which labor induces these changes is also unknown. It has been suggested that the increased circulating levels of catecholamines and cortisol associated with the stress of labor may be responsible for such immunomodulation (14). It is also possible that maternally derived labor-associated factors, such as IL-1, TNF, IL-6, IL-8, or prostaglandins may cross the placenta and mediate this effect. Other events associated with vaginal birth, such as extensive physical stimulation of the infant or brief hypoxemia, may play a role. Jokic and colleagues have recently reported increases in IL-6 and IL-8 in cord serum associated with fetal distress and labor independent of mode of delivery (18),

leading them to speculate that transient hypoxemia and acidosis during parturition was the cause. Alternatively, it may be that a factor or factors associated with cesarean birth, such as anesthesia, directly or indirectly inhibit IFN- γ production.

In summary, we found a significantly increased capacity of CBMC from infants experiencing labor to produce IFN- γ in response to both LPS and mitogens. Further studies are warranted to characterize the duration and mechanism of this effect.

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