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Skewed pattern of Toll-like receptor 4-mediated cytokine production in human neonatal blood: Low LPS-induced IL-12p70 and high IL-10 persist throughout the first month of life

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Abstract

Newborns are highly susceptible to infectious diseases, which may be due to impaired immune responses. This study aims to characterize the ontogeny of neonatal TLR-based innate immunity during the first month of life.

Cellularity and Toll-like receptor (TLR) agonist-induced cytokine production were compared between cord blood obtained from healthy neonates born after uncomplicated gestation and delivery (n=18), neonatal venous blood obtained at the age of one month (n=96), and adult venous blood (n=17). Cord blood TLR agonist-induced production of the Th1-polarizing cytokines IL-12p70 and IFN- α was generally impaired, but for TLR3, 7 and 9 agonists, rapidly increased to adult levels during the first month of life. In contrast, TLR4 demonstrated a slower maturation, with low LPS-induced IL-12p70 production and high IL-10 production up until the age of one month. Polarization in neonatal cytokine responses to LPS could contribute to neonatal susceptibility to severe bacterial infection.

Keywords

neonate; newborn; innate immunity; toll-like receptor; infection

Introduction

Neonates have an increased susceptibility to infection, causing significant morbidity and mortality. The incidence of infections is particularly high in the first weeks of life, and rapidly decreases thereafter². Common causes of infection in neonates include commensal bacteria such as group B streptococci and coagulase negative staphylococci, and Gram-negative organisms like *E. Coli*².

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Susceptibility to infection appears to be due to immaturity of the neonatal immune system. Neonatal adaptive immune responses are hampered by a lack of pre-existing memory and decreased Th1-type responses³. In addition, the innate immune system of newborns is also impaired⁴. Toll-like receptors (TLRs) are highly conserved components of the innate immune system and are involved in the recognition of microbial pathogen-associated molecular patterns. TLR activation triggers intracellular signalling cascades, resulting in production of inflammatory mediators that modulate the primary immune response and instruct the adaptive immune system. Thus, TLRs are essential in initiating and orchestrating the immune response. Studies of neonatal cord blood suggest that neonatal responses to multiple TLR agonists are impaired at birth. Neonatal cord blood monocytes demonstrate lower *in vitro* production of tumor necrosis factor- α (TNF- α) after stimulation with several TLR agonists, including bacterial lipopeptides (TLR2) and lipopolysaccharide (LPS; TLR4)^{5,6}. TLR-mediated responses in human cord blood dendritic cells (DC) are also distinct. Upon *in vitro* LPS stimulation, neonatal monocyte-derived DC (moDC) showed a significantly lower expression of activation markers CD40 and CD80 and decreased production of interleukin-12p70 (IL-12p70) and interferon- β (IFN- β) compared to adult moDC^{6,7}. Thus, impairments in the newborn TLR system may predispose for infections. The importance of the TLR system in newborns and infants is exemplified by patients with defects in the TLR-MyD88-IRAK4 pathway, who tend to present with severe infections early in life and clinical disease lessens with age^{8,9,10}.

Most studies assessing neonatal TLR responses used cord blood, which is more readily available than neonatal venous blood. However, the rapidly changing physiology at birth leads to significant changes to the blood compartment in the first hours and days of life. Because of the critical role of TLRs in the developing neonatal immune system, insight into the development of TLR function during the first months of life will likely contribute to a better understanding of the host defence against infection during this critical period in life. Here we show that unlike responses to agonists for TLR3, 7 and 9, neonatal responses to LPS are impaired throughout the first month of life, suggesting a TLR-pathway selective impairment that could contribute to susceptibility to particular infections.

Materials and Methods

Blood

The research protocol was approved by the local Medical Ethical Committee of the University Medical Centre Utrecht and written informed consent was obtained from parents of all participants. Blood was obtained from healthy newborns participating in an ongoing birth cohort study on the role of neonatal TLR responses in the pathogenesis of respiratory tract infections and asthma. Cord blood was collected directly after uncomplicated vaginal delivery (n=18). Peripheral venous blood was obtained by venipuncture at the age of 1 month (n=96), or from healthy adult volunteers (n=17). Exclusion criteria for blood collection at birth or at the age of one month were preterm delivery, a complicated obstetric history, perinatal use of antibiotics by mother or child or any type of medical intervention. To investigate the timing of TLR4 maturation, a third group of children was included from whom venous blood was collected 5 days (range 1-7) after delivery (n=22). In the latter group, we allowed for minor medical issues, such as macrosomy or low temperature warranting glucose control. None of the participants had any sign or symptom of infectious disease, such as respiratory tract complaints or fever, in the two weeks prior to sampling. Due to practical considerations, we were unable to obtain repeated blood samples in the same children. Baseline characteristics are shown in Table 1. Blood was collected in sterile tubes and anticoagulated with EDTA for differential blood count, or with sodium heparin for flow cytometry and *in vitro* TLR stimulation assays. Limited blood volume and technical issues prevented us from performing

all measurements in all subjects. The exact N for each experiment can be found in supplementary table 1.

Flow cytometry

Expression of cell surface antigen was determined by incubating whole blood samples with fluorescence-labeled monoclonal antibodies for 15-30 minutes. Antibodies were conjugated to fluorescein isothiocyanate (FITC) (CD8, CD14, CD45RA, CD56, lineage cocktail), phycoerythrin (PE) (CD5, CD16, CD45RO, CD62L, CD123), allophycocyanin (APC) (CD3, CD11c, CD19) or peridinin-chlorophyll-protein complex (PerCP) (CD4, HLA-DR). All antibodies were obtained from Becton and Dickinson Biosciences, Franklin Lakes, NJ.

After incubation, red blood cells were lysed using $1\times$ lysing solution (BD Biosciences). Cell pellets were washed in phosphate-buffered saline and fixed using 1% paraformaldehyde. Flow cytometry was performed using the FACS Calibur system (BD Biosciences) and data were analyzed using CellQuest pro software (BD Biosciences). Whole blood concentrations of lymphocytes and neutrophils were determined by total and differential leukocyte count using the Cell-Dyn Sapphire haematology analyzer (Abbott diagnostics, Abbott Park, IL). Manual leukocyte differential was performed in case of abnormal cell morphology. Myeloid dendritic cells (mDC) were identified as HLA-DR⁺, lineage⁻ and CD11c⁺, plasmacytoid dendritic cells (pDC) as HLA-DR⁺, lineage⁻ and CD123⁺. Natural killer (NK)-cells were marked by CD3⁺, CD16 and CD56⁺, and monocytes were identified as HLA-DR⁺ and CD14⁺. Absolute numbers of mononuclear cells were calculated by multiplying the percentage of cells in the lympho-monocyte gate (as determined by flow cytometry) with the concentration of lymphocytes and monocytes from the differential leukocyte count.

TLR agonists

TLRs were stimulated using polyinosinic:polycytidylic acid (poly I:C, TLR3), ultrapure LPS from *Escherichia Coli* (TLR4), loxoribine (TLR7) and CpG oligonucleotide type A (ODN CpG 2216, TLR9), all from InvivoGen (San Diego, CA). For co-stimulation, recombinant IFN- γ was purchased from PeproTech Inc. (Rocky Hill, NJ).

Cell stimulation

In vitro TLR stimulation was performed using optimal concentrations of TLR agonists and incubation times for cytokine measurements, as titrated in pilot experiments (data not shown). Accordingly, blood samples were stimulated with LPS (100 ng/ml) + IFN- γ (20 ng/ml) poly I:C (200 μ g/ml), ODN CpG (30 μ g/ml) or loxoribine (1 mM). For mononuclear cell stimulation in plasma exchange assays, lower concentrations of stimuli were used (50 ng/ml LPS and 20 ng/ml IFN- γ). For cytokine protein measurements in culture supernatant, blood samples were diluted 1:14 in RPMI medium containing 2.0 mM glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin prior to *in vitro* TLR stimulation. This dilution allowed us to study cytokine responses to multiple TLR agonists in limited blood volume. After 24h incubation at 37°C and 5% CO₂, samples were centrifuged at $1000\times g$ for 5 min. Supernatants were collected and stored at -80°C until further analysis. For RNA studies, stimulations were performed in undiluted blood using a 5h incubation time optimized for RNA detection. Upon stimulation, blood was collected in PAXgene reagent (PreAnalytiX GmbH, Hombrechtikon, Germany) and stored at -80°C until further processing.

Plasma studies

The effect of plasma on TLR-agonist induced cytokine production by PBMC was studied according to previous reports⁶. Plasma was prepared by centrifugation of heparinized blood at 1000 g for 10 minutes. Fresh adult peripheral blood mononuclear cells (PBMC) were obtained

by Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden) gradient separation, and stimulated with LPS (50 ng/ml) and IFN- γ (20 ng/ml) in the presence of 10% heterologous adult or neonatal plasma (24 h; 37°C; 5% CO₂). For each experiment, plasma derived from 8-16 different one-month old neonatal or adult donors was used.

Cytokine ELISA

Cytokine concentrations in culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions: IL-10 (Sanquin/CLB, Amsterdam, The Netherlands), IL-12p70 (Diaclone Research, Besançon, France) and IFN- α (Bender Medsystems, Burlingame, CA, USA). Internal controls were used to minimize inter-assay variations. The lower limits of detection were 1.0 pg/ml (IL-10), 2.0 pg/ml (IL-12p70) and 2.7 pg/ml (IFN- α). For samples in which the cytokine concentration was below the detection limit, the concentration was arbitrarily defined as half of the detection limit.

RNA measurements

RNA was extracted using the PAXgene Blood RNA kit (PreAnalytix GmbH, Hombrechtikon, Germany), according to a modified protocol optimized for small blood volumes (ref Carrol BMC Immunology 2007). RNA was subsequently purified and concentrated using the RNAeasy mini-elute kit (Qiagen, Valencia, CA) according to manufacturer's instructions. RNA concentrations in the purified samples were measured using a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and cDNA was prepared using the RT² First Strand Kit (SA Biosciences, Frederick, MD). qRT-PCR was performed according to manufacturer's instructions, using a customized PCR array including 28 different TLR-related transcripts (SA Biosciences). SYBR-Green (SA Biosciences) was used for detection and fluorescence was read on the ABI Prism 7300 Sequence Detector (Applied Biosystems, Foster City, CA). Resulting mRNA levels were normalized to housekeeping genes and compared using the Δ CT method.

Statistical analysis

All data were analyzed in the Statistical Package for Social Sciences (SPSS) version 15.0 software. The distribution of variables was checked for normality using the Kolmogorov-Smirnov test. Cytokine and mRNA concentrations after TLR stimulation and flow cytometry data were logarithmically transformed, and geometric means between groups were compared using Student's *t* test, or one-way ANOVA with post-hoc analysis (Bonferoni test for multiple comparisons). Correlations between LPS-induced and LPS+IFN γ -induced release of IL-10 and IL-12p70 were calculated using Pearson correlation on logarithmically transformed data. All *p* values are two-sided and were considered significant when *p* < 0.05.

Results

Different cell composition in neonatal and adult venous blood

To determine whether differences in innate immune cell numbers may contribute to the pattern of neonatal TLR-mediated responses, whole blood leukocyte differentials were performed by flow cytometry. Cord blood and neonatal venous blood were highly cellular compared to adult venous blood, containing higher concentrations of monocytes (figure 1A) and equal concentrations of mDC, pDC and natural killer cells (figure 1B-D). Consistent with previous studies, neutrophil concentrations at the age of one month were lower than those in cord blood and adult blood (figure 1E)¹¹.

Cord blood TLR agonist-induced cytokine release is distinct

To assess the ability of neonatal innate immune cells to mount an immune response to microbial products, we tested *ex vivo* whole blood responses to a panel of TLR agonists. Cytokine concentrations in unstimulated control samples were below the limit of detection for all cytokines (data not shown). Cord blood TLR agonist-induced cytokine responses were significantly different from adult TLR responses (figure 2 and 3). Cord blood hemocytes demonstrated similar (TLR3 and TLR7) or increased (TLR4 and TLR9) levels of TLR agonist induced IL-10. In contrast, cord blood production of Th1-polarizing cytokines IL-12p70 and IFN- α in response to agonists for TLR3, TLR4 and TLR7 was decreased compared to adults.

TLR agonist-induced cytokine responses differentially mature during the first month of life

To determine whether the distinct patterns of neonatal TLR-mediated responses persist beyond the immediate peripartum period, we next determined TLR agonist induced cytokine release in healthy newborns at the age of one month. Neonatal capacity to produce Th-1 type cytokines IL-12p70 or IFN- α in response to agonists of TLR3, TLR7 and TLR9 rapidly increased to adult levels within the first month of life (figure 2). In marked contrast, TLR4-mediated production of IL12p70 remained significantly decreased compared to adults (geometric mean 47 pg/ml, 95%-CI 29-76 pg/ml vs 437 pg/ml, 166-1129 pg/ml, $p < 0.05$), (figure 3). 1 month old neonates demonstrated increased production of IL-10 in response to stimulation with agonists for TLR3, TLR4, TLR7 and TLR9. However, although differences in TLR3-mediated IL-10 production were significant, these results should be interpreted with caution because poly I:C induced very little IL-10 in all populations. At age one month, there was a modest inverse correlation between TLR4-mediated IL-10 and IL-12p70 production ($\rho = -0.167$, $p < 0.05$).

TLR-4 responses gradually mature during the first month of life

To study whether neonatal TLR4-mediated cytokine responses are impaired all throughout the first month of life, LPS+IFN- γ -induced cytokine production was studied in venous blood obtained from children during the first week of life (figure 3). LPS+IFN- γ stimulation induced similar amounts of IL-10 in cord blood (1192 pg/ml), venous blood drawn at the age of one week (741 pg/ml) and at the age of one month (988 pg/ml). LPS+IFN- γ -induced IL-12p70 production at age one week was significantly higher than in cord blood (53 pg/ml vs 6.5 pg/ml, $p < 0.01$), similar to levels at age one month (47 pg/ml, pg/ml), and lower than in adults (433 pg/ml, $p < 0.01$). These findings suggest that the ability of neonatal blood to produce Th-1 type cytokines in response to LPS remains impaired at least until the age of one month. Previous studies have shown that perinatal events influence cord blood TLR agonist induced cytokine production^{6,12}. In our study, both univariate and multivariate analyses did not reveal any association between gestational age, sex, maternal parity or mode of delivery and TLR agonist induced cytokine production in neonatal venous blood drawn at the age of one month (data not shown). This study was not sufficiently powered to investigate the clinical determinants of neonatal TLR responses in cord blood or venous blood at the age one week.

Distinct neonatal responses to LPS are associated with differences in cytokine transcription

We next examined whether the distinct neonatal TLR4-mediated IL-12p70 and IL-10 protein responses are also evident on mRNA level (figure 4). Stimulation with LPS or LPS+IFN- γ induced high levels of IL-12A (p35) mRNA in adults. In contrast, cord blood and neonatal venous blood demonstrated impaired LPS-induced up regulation of IL-12A mRNA (figure 4A), with significantly lower levels of LPS-induced IL-12A compared to adults. Although not significant, IL-12B demonstrated similar patterns (figure 4B). Suggesting that differences in mRNA levels may underly the observed differences in LPS-induced IL-12p70 protein production between neonates and adults.

In contrast, despite higher LPS-induced IL-10 protein in cord blood (figure 3), LPS-induced levels of IL-10 mRNA were similar in all age groups (figure 4E). However, small but significant differences may have remained undetected because of a relatively small number of participants (7 per group) for this part of the study.

As previous studies on neonatal TLR responses mainly focused on TNF- α and IL-6^{5,6,13}, we determined LPS- and LPS+IFN- γ -induced mRNA levels of these cytokines. Consistent with existing literature, TLR4-mediated production of TNF- α mRNA trended lower in cord blood and at the age of one month compared to adults (figure 4C), whereas TLR4-mediated transcription of IL-6 was similar in all age groups.

Effect of recombinant IFN- γ on LPS-induced cytokine production

Stimulation with LPS only (i.e. without IFN- γ) resulted in low/undetectable levels of IL-12p70 in 77% of all samples. Addition of exogenous IFN- γ has been shown to increase transcription of IL-12p35 and IL-12p40, and to increase LPS-induced release of IL-12p70^{14,15}. Therefore, recombinant IFN- γ (rIFN- γ) priming was used to optimize LPS-induced IL-12p70 production. To verify that the observed patterns were not solely the result of a newborn-specific effect of IFN- γ , we compared cytokine production in samples stimulated with LPS only and LPS with IFN- γ (supplementary figure 1). Production of IL-12p70 showed similar developmental patterns, with low/undetectable levels at birth and higher levels at the age of one month or in adults. Interestingly, although there was a strong correlation between LPS-induced and LPS+IFN- γ -induced IL-10 production ($\rho=0.809$), LPS-only stimulation resulted in high-levels of IL-10 with no differences between age groups. This suggests that the effect of IFN- γ may be age-dependent, selectively inhibiting LPS-induced IL-10 production in one-month olds and adults, but not in cord blood.

Neonatal LPS-induced production of IL-10 but not IL-12p70 is influenced by a soluble factor

The maturation lag of TLR4 responses prompted us to further explore the underlying mechanism of distinct neonatal LPS-induced cytokine production. To determine whether differences in TLR4 complex-mediated production of IL-10 and IL-12p70 are due to cellular or soluble factors, we compared LPS-induced IL-10 and IL-12p70 release from adult PBMC cultured in heterologous adult and neonatal plasma obtained at the age of one month (figure 5). Neonatal plasma conferred diminished release of IL-10 in two out of three PBMC donors ($p<0.05$), but did not affect IL-12p70 release. The magnitude of modulation of IL-10 release by plasma was smaller than the differences observed in our whole blood assay. In addition, the donor-specificity and lack of plasma-mediated effect on IL-12p70 release suggest that differences in neonatal and adult LPS-induced cytokine production may not be solely modulated by soluble factors.

Discussion

Human neonates are highly susceptible to infections, which is generally ascribed to transient developmental deficiencies in the innate and adaptive immune system. Here, we demonstrate that despite similar or higher concentrations of innate immune cells, neonatal ability to produce Th1-type cytokines upon TLR stimulation is impaired compared to adults. The neonatal TLR system undergoes rapid and differential development during the first month of life. Whereas the ability to produce Th1-type cytokines in response to agonists for TLR3, TLR7 and TLR9 rapidly increases to adult levels during the first month of life, TLR4 mediated responses remain impaired at least up to one month of age. Age-dependent impairments in the TLR system may contribute to the high neonatal susceptibility to infection. Our results are consistent with previous studies, demonstrating that neonatal innate immune system is distinct and polarized towards Th-2-type responses^{6,7,16}. This polarization is thought to play an important role in the

prevention of harmful maternal-fetal alloimmune reactions leading to preterm labour and delivery⁵. However, the bias against Th-1-cell-polarizing cytokines leaves the newborn susceptible to infection. Insight into the kinetics and factors modulating neonatal TLR development may result in new strategies to prevent and/or treat infections and allergic diseases.

Studies investigating postpartum TLR development describe gradual maturation of Th-1 polarizing capacity from infancy to childhood^{12,17,18}. The importance of establishing the immune status in early life is underscored by several studies showing that variations in early immune development have long-term sequelae with regard to the prevalence of many diseases¹⁹⁻²². To our knowledge, this is the first study to investigate postpartum TLR development during the first month of life in a large cohort of healthy newborns. The striking maturation of neonatal TLR responses identifies the first month of life as an essential period in the development of this part of the innate immune system.

Studies investigating the cause of impaired neonatal TLR responses have mainly focused on impaired production of Th1-type cytokines such as TNF- α and IL-12^{4,6,7,13,23,24}. Different mechanisms have been suggested to explain decreased cord blood LPS-induced IL12p70 production. Firstly, plasma factors, such as LPS binding protein have been shown to modulate LPS-delivery to the TLR4 receptor complex^{25,26}. Adenosine, present in neonatal plasma, has been shown to increase intracellular levels of cyclic adenosine monophosphate (cAMP), thereby inhibiting TLR-mediated TNF- α production¹³. Interestingly, increased levels of cAMP also decrease IL-12 production while increasing production of IL-10, exemplifying its immunopolarizing potential⁵. In our assay, neonatal plasma showed a modest increase in LPS-induced release of IL-10 in two out of three adult PBMC donors, and had no effect on the production of IL-12p70. Because of limited volume, we were only able to use 10% plasma, whereas previous studies used 100% plasma. In addition, instead of cord blood mononuclear cells, we used adult PBMC, which may be less sensitive to the TLR-modifying effects of neonatal plasma. We hypothesize that TLR4-mediated cytokine production is modulated by a complex and dynamic interplay between soluble factors and other (cell intrinsic) factors. The concentration of soluble mediators and target cell sensitivity will be subject of further studies. Secondly, differences in LPS-induced cytokine production may be due to differences in expression of the TLR4 complex, consisting of TLR4, CD14 and MD2. The relative level of TLR4 expression in neonates compared to adults is still a matter of debate, which is further complicated by methodological differences between studies^{6,18,27}. However, while differences in TLR4 complex expression or soluble factors may explain a general increase or decrease in cytokine release, they are unlikely to explain the polarization observed in our study.

Thirdly, IL-10 has been shown to negatively modulate IL-12p70 production^{28,29}. Indeed, in our samples, there was a modest, but significant negative correlation between LPS+IFN- γ induced IL-10 and IL-12p70 release. This finding supports the notion that TLR4-mediated cytokine production is tightly regulated to maintain the balance between Th1 and Th2. From our results, we cannot conclude whether polarized neonatal TLR4 responses are primarily due to increased production of IL-10, decreased production of IL-12p70, or to a common factor influencing both cytokines.

Fourthly, signalling downstream of TLR4 also differs between newborns and adults. A microarray study comparing cord blood and adult LPS-activated monocytes showed significant differences in expression of several signal transduction factors and transcription factors, including JunB and STAT4³⁰. LPS-induced mRNA levels of both subunits of the IL-12p70 heterodimer by cord blood monocytes has been shown to be decreased due to decreased half-life (IL-12p40) or defective nucleosome remodelling, resulting in impaired transcription (IL-12p35)³¹⁻³⁴. Our data indicate that, in a whole blood assay, defective IL-12p70 production

at birth is due to impaired transcription of both IL-12A (encoding the IL-12p35 subunit) and IL-12B (IL-12p40), and that these impairments are maintained up until the age of one month. This study has potential limitations. Firstly, *in vitro* study of whole blood represents a minimally perturbed system, yet may not reflect patterns of response *in vivo*³⁵. Secondly, repeated sampling in the same children would have been the optimal strategy to address maturation of TLR responses during the neonatal age. This was not feasible for practical reasons. Thirdly, the whole blood stimulation assay limits conclusions on cell-specific mechanisms underlying impairments in neonatal TLR function. Similar limitations would have been encountered using isolated PBMC, because PBMC composition changed markedly during the neonatal age. We believe that the whole blood stimulation model is a relevant representation of the immunological status of newborns and infants that correlates with susceptibility to infection³⁶⁻³⁸.

In conclusion, neonatal TLR responses are distinct from those of adults, and differentially mature during the first month of life. The propensity towards high IL-10 but low IL-12p70 production in response to TLR4 agonists during the first month of life might contribute to neonatal susceptibility to pathogens that are recognized by TLR4, such as Gram-negative bacteria and RSV³⁹. Finally, future studies aimed at identifying the factors influencing postpartum TLR development will further delineate the age-dependent maturation of this key aspect of innate immunity and may identify new strategies to modulate the maturation of the neonatal innate immune system, to prevent infections and/or allergy during this vulnerable age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Whole blood concentrations of innate immune cells

Whole blood leukocyte differential was performed in cord blood (n=18), neonatal venous blood obtained at the age of one month (n=96) and adult venous blood (n=17). Cell percentages were determined by FACS and multiplied by the total concentration of lymphocytes and monocytes from the complete blood count. Data are represented as geometric mean + 95% CI. *; $p < 0.05$.

Figure 2. Rapid maturation of TLR responses in healthy newborns

Cytokine production in cord blood (n=18), neonatal venous blood at age one month (n=96) and adult venous blood (n=17) was measured after a 24h-incubation with poly I:C (TLR3, panel A+B), Loxoribine (TLR7, C+D) and ODN CpG (TLR9, E+F). Neonatal ability to produce Th1-type cytokines upon TLR-agonist stimulation was impaired at birth, but rapidly increased to adult levels. Data are represented as geometric mean + 95% CI. *, $p < 0.05$.

Figure 3. Distinct TLR4 responses up until the age of one month

Cytokine production in cord blood (n=18), neonatal venous blood at age one month (n=96) and adult venous blood (n=17) was measured after a 24h-incubation with LPS+IFN- γ . Cord blood TLR4 responses were characterized by high levels of IL-10 (A) and low levels of IL-12p70 (B). Although LPS+IFN- γ -induced production of IL-10 and IL-12p70 gradually decreased or increased respectively, TLR4-mediated cytokine responses at the age of one month remained significantly impaired compared to adult responses. *, $p < 0.05$.

Figure 4. Human neonatal cord blood demonstrates reduced LPS-induced cytokine mRNA responses compared to adults

Cytokine mRNA concentrations in cord blood (n=7), neonatal venous blood at age one month (n=7) and adult venous blood (n=7) were measured after a 5h-incubation with medium, LPS or LPS+IFN-γ. TLR4-mediated transcription of IL-12A and IL-12B and TNF-α was impaired both in cord blood and in venous blood at age one month, compared to adult venous blood. In contrast, despite higher medium-induced levels in cord blood, TLR4 agonist-induced production of IL-6 and IL-10 was similar in all age groups. *, $p < 0.05$.

Figure 5. Donor- and cytokine specific effect of neonatal plasma on LPS-induced cytokine production

Adult PBMC derived from heparinized blood from three separate donors were stimulated with LPS and IFN- γ in the presence of 10% heterologous adult or neonatal plasma. Each dot represents a different plasma donor. After 24h incubation, the extracellular medium was collected for IL-10 and IL-12p70 measurements. Bars represent median values.

Table 1

Characteristics of participants

Characteristic	Group		
	Birth (n=18)	1 wk (n=22)	1 mo (n=96)
Gender, male, <i>n</i> (%)	10 (56)	13 (59)	49 (51)
Gestational age, wk, mean (95% CI)	40.0 (39.3-40.6)	39.9 (39.3-40.6)	39.8 (39.5-40.0)
Birth weight, g, mean (95% CI)	3650 (3443-3857)	3643 (3354-3931)	3583 (3487-3678)
Mode of delivery, <i>n</i> (%)			
Vaginal	18 (100)	6 (27)	67 (70)
Cesarean section	0 (0)	16 (73)	29 (30)
Siblings, <i>n</i> (%)	13 (72)	16 (73)	59 (61)