Pediatric RESEARCH

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BASIC SCIENCE ARTICLE

Ontogeny of cytokine responses to PHA from birth to adulthood

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BACKGROUND: Altered production of cytokines is believed to contribute to early childhood susceptibility to infection. The aim of this study was to get further insight into the developmental patterns of cytokine responses from birth to adulthood.

METHODS: The expression levels of 13 cytokines were compared in the supernatants of phytohemagglutinin (PHA)-stimulated whole blood from healthy neonates (cord blood, $n = 8$), infants (< 1-year–old, $n = 20$), and school-aged children (3–15 y; $n = 20$). Five adults were used as reference.

RESULTS: While Th1, Th2, and Th17 cytokine levels increased progressively from birth to childhood (Mann–Whitney, $p < 0.003$), high IL-10 secretion at birth dropped to low adult levels in infants ($p < 0.004$) such that a negative correlation between IL-10 and Th1, Th2, and Th17 cytokine levels at birth (Spearman's correlation, $r < -0.70$, $p < 0.01$) converted to a positive correlation in infants ($r > 0.60$, $p < 0.001$). Finally, high IL-2, IL-7, and Granulocyte-Colony Stimulating factor (G-CSF) cytokine levels at birth decreased steadily over the first year of life (Mann–Whitney, $p \leq 0.001$).

CONCLUSION: The most noticeable result of the study is the rapid shift from enhanced IL-10 secretion capacity at birth toward balanced IL-10/Th1/Th2/Th17 cytokine levels early in life. This change appears an essential precondition to fight pathogens and at the same time to avoid overwhelming inflammatory reactions.

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INTRODUCTION

Despite advances in medicine, the morbidity due to severe infection during infancy remains a major global concern even in industrialized countries. Besides increased susceptibility to infectious diseases, a decrease in vaccine efficacy is often observed in early age.

Immune responses are tightly regulated by multiple factors including cytokines that are secreted by both adaptive immune cells (B and T-cells) and innate immune cells (among which monocytes macrophages and dendritic cells).

Although altered production of cytokines is believed to be an important contributing factor to childhood susceptibility to infection[1], contrasting results with regard to the capacity of neonatal immune cells to secrete cytokines limit our current understanding of the development of cytokine expression in childhood. Enormous heterogeneity in the study design has been cited as a major cause of result variability especially with regard to the development trajectory of adaptive cytokines[2].

T-cells from the adaptive compartment are grossly divided into effector cells and regulatory cells. The effector cells can be subdivided into several types including Th1, Th2, and Th17 cells. Th1 immune cells promote immunity to intracellular pathogens through IFN-γ and TNF-α secretion. Th2 immune cells promote immunity to parasites and regulate Ig production by cells through IL-4, IL-5, and IL-13 secretion. The more recently discovered Th17 cells promote immunity to bacterial and fungal infections at epithelial surfaces. Th1, Th2, and Th17 cells have substantial interaction and cross-regulation and what type of effector cell that dominates an immune response will have major implications for the capacity to eliminate a pathogen.

Impaired ability to mount a Th1 response at birth was clearly demonstrated by deficient IFN-γ and TNF-α cytokine expression by cord T-cells following polyclonal activation[3–7]. Also, Th1 responses to a number of vaccines and infectious pathogens are poor during early life[8]. The innate immune system, known to instruct the adaptive immune system[9] likely contributes to defective Th1 cytokine expression. Defective secretion by macrophages of IL-12, a key Th1-polarizing cytokine has been known for a long time[10]. This observation was complemented over the past decade by a growing number of studies showing reduced Th1-polarizing cytokine secretion by innate immune cells in response to stimulation through Toll-Like Receptors (TLRs) in newborns[11].

The concept of a general Th2 bias in newborn mice has been extended to the immune system of the newborn child. Support of this proposal comes from some human studies that investigated infant responses to vaccines[1,12,13]. Other studies are not in agreement. Overall, the capacities to produce both Th1 (IFN-γ) and Th2 (IL-4, IL-5) cytokines by immune cells following polyclonal T-cell activation were found to be suppressed to approximately the same degree in newborns compared with adults in most studies[3–7,14,15].

Studies on Th17 maturation in early life are scarce. Overall, newborn Th17 cells appear defective in their ability to secrete IL-17[14] unless supplemented with Th17-polarizing cytokines IL-1 beta, IL-6 and IL-23[16].

The regulatory cells function to suppress immune responses. IL-10 is an anti-inflammatory cytokine that plays a critical role in protecting the host from tissue damage during acute phases of immune responses. IL-10 was initially identified as a Th1 inhibitory factor

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secreted by Th2 cells[17]. Later on, T-cells producing IL-10 but not IL-4 differentiated them from Th2 cells[18]. Nowadays, IL-10 is known to be produced by virtually all innate and adaptive immune cells[19]. Although a propensity towards high IL-10 production by innate immune cells in response to several TLR agonists at birth is largely documented[20] not much is known about the capacity of newborn T-cells to produce IL-10. Several studies using T-cell-specific (anti-CD3 monoclonals) polyclonal activators or T-cell mitogen reported reduced IL-10 production[21–24], but however others reported increased IL-10 secretion capacity by neonatal T-cells[25].

Most studies exploring cytokine secretion capacity in young age used cord bloods as source of immune cells. Studies addressing the ontogeny of cytokines from birth to adulthood are more limited. The aim of this study was to perform a comprehensive analysis of the evolution of cytokine profiles after birth. We conducted a cross-sectional observational study including neonates, infants, and school-aged children. A multiplex panel for the detection of multiple cytokines including the Th1 (IFN-γ, TNF-α), Th2 (IL-4, IL-5, IL-13), Th17 (IL-17) cytokines implicated in effector immune functions; the IL-1 beta, IL-6, and IL-12 pro-inflammatory cytokines; the IL-10 immunosuppressive cytokine; and the IL-2, IL-7, and Granulocyte-Colony Stimulating Factor (G-CSF) regulatory/homeostatic cytokines was used to measure cytokines in supernatants of whole blood stimulated with phytohemagglutinin (PHA), a potent T-cell activator. To circumvent the difficulty in the availability of blood samples from young healthy children, residual supernatants from peripheral blood activated ex vivo by PHA for diagnosis purpose were used.

MATERIAL AND METHODS

Study subjects and blood sampling

To prevent blood drawing in healthy children specifically for the current study, we restricted our study population to healthy newborns (cord blood) and to those individuals who had to undergo a medically indicated QuantiFERON-TB Gold In-Tube test (Cellestis Ltd Qiagen Chadstone VIC Australia) within the scope of screening tuberculosis infection that was subsequently excluded. In those individuals, residual plasmas from the test were used for this study. Prior to each blood draw, informed consent was obtained from mothers before birth (cord blood), from parents (infants, school-aged children), and from adults. The study was approved by the Robert Debré Hospital institutional ethics review board.

Blood sampling was performed according to the recommendations of the quantiFERON test manufacturer and as previously described[26] in all cases and processed by the same experimented staff during the study period.

Four age groups were selected. These included newborns (cord blood) from uncomplicated and full-term pregnancies ($n = 8$), infants aged less than 1-year-old ($n = 20$), school-aged children ($n = 15$), and a group of healthy adults from the staff of the department ($n = 5$).

Exclusion criteria for cord blood selection were preterm delivery, a complicated obstetric history, and any type of medical intervention in mother or child.

Exclusion criteria for the selection of older individuals were an ongoing or recent history of infection, medication, or drug administration deemed to affect immune competencies, malnourishment, the risk of HIV-infection, and any known or suspected immune deficiency.

Whole blood stimulation

Whole blood stimulation was performed using the QuantiFERON kit according to the manufacturer's instructions. Briefly, one milliliter of blood was taken and incubated in each of three tubes: one pre-coated with Mycobacterium tuberculosis-antigens (test tube), the second pre-coated with Phytohemagglutinin (PHA positive control tube), and the third pre-coated with saline (negative control tube). The tubes were incubated for 16–24 h at 37 °C, 5% atmospheric CO_2, and the supernatant was collected after centrifugation. After exclusion of tuberculosis infection, residual supernatants (plasma) were stored at −80 °C until the assay was done. For this study, we considered the supernatants of the unstimulated blood (negative control tube) and the PHA-stimulated blood (positive control tube) to perform cytokine measurements.

Cytokine measurement in residual supernatants of whole blood cultures

Plasma samples were tested using a multiplex cytokine assay (Bio-Plex Human Cytokine 17-Plex Panel; Bio-Rad Laboratories Inc., Marnes-la-coquette, France) containing assays for interleukin IL-1 beta, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-10, IL-12(p70), IL-13, IL-17, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ, macrophage chemoattractant protein 1 (MCP-1)/CCL2, macrophage inflammatory protein 1 beta (MIP-1b/CCL4), and tumor necrosis factor (TNF-α) as previously described[27]. Cytokine levels were analyzed on a Luminex Analyser using Bio-Plex Manager 6.0 (Bio-Rad Laboratories) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation were <12% for all analytes. Four of the markers evaluated that were either at the higher (IL-8, MCP-1, and MIP-1b) or at the lower [IL-12(p70)] level of detection in all samples were excluded from further statistical analysis.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0. Clinical parameters between the considered groups were compared using Fisher exact statistical test. The general significance level was set to 0.05. The median values of each cytokine from the PHA-stimulated tube was determined and compared between the groups by using the Mann–Whitney *U* test. Spearman's correlation analysis was carried out to determine the association of supernatant cytokine levels either with age or between the capacities of production of the different cytokines intrinsically. Taking into account effects of multiple testing, we applied the Bonferroni adjustment, which sets a marginal significance level of 0.0038 (corresponding to Bonferroni adjustment for 13 tests).

RESULTS

Main characteristics of the study population

The main characteristics of the study population according to age are given in Table 1. None had comorbidity according to selection criteria. As expected from the vaccination schedule, differences with regard to vaccine status were observed between age groups. Information on BCG-vaccination that has a number of non-specific immunological effects in addition to protection against tuberculosis[28] is given in Table 1.

Age-related changes in cytokine secretion capacity

As shown in Table 2, 13 cytokines were clearly induced by PHA. Of note, IL-7 was clearly induced by PHA in neonates, while trends toward higher concentration levels in the PHA-tube than in the unstimulated tube (background) did not reach significance in the other clinical groups. Although background levels of these cytokines in the unstimulated blood samples (background) were highly similar in the four clinical groups (Table 2), the concentration levels of IFN-γ, TNF-α, IL-13, IL-5, IL-17 cytokines (Fig. 1), and of IL-2, IL-17, G-CSF and IL-10 cytokines (Fig. 2) were critically age-dependent in the PHA tubes.

Low IFN-γ release was recorded in PHA-stimulated cord blood opposed to infants ($p = 0.0007$). IFN-γ levels gradually increased with aging to reach adult levels in school-age children. Overall, we detected a similar developmental pattern for IFN-γ and for all other Th1, Th2, or Th17 cytokines (Fig. 1).

Table 1. Children characteristics in the 3 groups of age

Characteristics	Neonates (cord bloods) ($n = 8$)	Infants ($n = 20$)	p^a	School-aged children ($n = 15$)	p^b
Age					
Median (range), d, m, y	0	6 m (15 d–11 m)	**Irrelevant**	5 y (3 y–15 y)	**Irrelevant**
Sex					
F/M, *n/n*	3/4	11/9	0.67	7/8	0.73
BCG-vaccination, *n*					
Yes	0	14	**0.001**	15	**0.02**
No	8	6		0	
Associated diseases, *n*					
None	8	20	1	15	1
Unknown	0	0		0	
Estimated Term of birth[c]					
Median (range)	39 (39–40)	39 (35–40)	0.44	ND	—
Mode of delivery					
Vaginal birth, *n*	8	19	1	15	1
Cesarean, *n*	0	1		0	
Birth weight (Kg)					
Median (range)	3.25 (2.98–3.78)	3.09 (2.50–4.00)	0.62	ND	—

p-values below 0.05 thresholds are considered significant using Fisher's exact test for qualitative parameters and the Mann–Whitney test for quantitative parameters and are highlighted in bold
d days, *m* month, *y* year
[a]Test comparing infants to neonates 1
[b]Test comparing infants to school-aged children
[c]Week gestation

In contrast, IL-2, IL-7, and G-CSF cytokine concentrations in PHA-stimulated blood samples declined with age, with the highest levels found in neonates (cord bloods). Regarding the immunosuppressive IL-10 cytokine, PHA-stimulation induced production of IL-10 in cord bloods at levels far above those found in infants, which then stabilize (Fig. 2).

A high inter-individual heterogeneity in cytokine response intensities was observed after birth, especially among infants. Some infants evidenced cytokine levels comparable to adults, while others showed lower cytokine production. An influence of BCG-vaccination on this variability was not obvious in BCG-vaccinated versus non-vaccinated infants ($p > 0.23$).

Finally, background and PHA-induced levels of IL-6 were similar in the four clinical groups and trends toward a slow decrease in background and PHA-induced IL-1 beta production with aging did not reach significance.

Age-related changes during the first year of life

During the first year of life, no significant change in the production intensity of Th1, Th2, and Th17 cytokines with aging was observed (data not shown). On the contrary, as illustrated in Fig. 3, a progressive decrease in the release of IL-7 and of G-CSF was observed from 15 days to 11 months (350 days) of age. A similar trend was observed for IL-2. Finally, IL-10 secretion capacity dropped from neonatal to adult values (Fig. 2) soon after birth (Fig. 3) with no significant change from 15 days of age (the youngest infant we explored) to 11 months of age (the oldest infant we explored).

Age-related changes in cytokine network

It is well established that cytokines interact in a complex network. We thus studied the correlations of production intensity between any cytokine for a better characterization of response patterns depending on age.

A positive correlation was observed between the levels of Th1, Th2, or Th17 cytokines, irrespective of age (not shown). In contrast, IL-10 levels were negatively correlated with Th1, Th2, and Th17 cytokine levels in neonates ($r < -0.70$) whereas among infants and school-age children, IL-10 levels were positively correlated with Th1, Th2, and Th17 cytokine levels ($p > +0.60$). It is worth noting that the positive correlation between IL-10 and Th1, Th2 and Th17 cytokine levels occurred soon after birth, as demonstrated by critically lower IL-10 over Th1, Th2 and Th17 cytokine ratios in the youngest infant (2 weeks of age) opposed to any neonate. Figure 4 illustrates these changes by showing a correlation between IL-10 and selected Th1 (IFN-γ), Th2 (IL-13) Th17 (IL-17) prototypes according to age group.

DISCUSSION

Although data on the ontogeny of cytokines present a degree of heterogeneity, most authors found, in line with our results, that impaired Th1 and Th2 cytokine expression upon stimulation with polyclonal activators progressively increases from birth to adult levels during childhood[3,5,29–31]. The concept of Th2 bias in early life is more controversial.

In agreement with other authors who used polyclonal activators for the calculation of Th1/Th2 cytokine ratios at birth and/or over time[3–7,14,15,32], we did not observe a Th2 bias in newborn or infant groups. These observations suggest that a Th2 bias is not inevitable. In line with this hypothesis, infants administered BCG or whole-cell pertussis vaccines had immune response similar to adults[33,34], despite the well-know Th2 biased response by neonatal or infant immune cells to several vaccines or infectious agents[12,13,32,35–37]. Also, several experimental data showed that mature adult like immune responses can be developed under appropriate conditions of neonatal T-cells stimulation[38].

Table 2. Cytokine levels (pg/ml) according to age

Cytokine[a]	Cord bloods			Infants			School-aged children			Adults		
	Background[b]	PHA[b]	*P*	Background[b]	PHA[b]	*P*	Background[b]	PHA[b]	*P*	Background[b]	PHA[b]	*P*
IL-2	88 (55–170)	2732 (2332–3143)	**0.0001**	152 (71–299)	412 (169–3834)	**0.0003**	103 (61–915)	414 (203–2766)	**0.0001**	60 (38–163)	529 (121–4454)	**0.0001**
IL-5	17 (11–48)	41 (27–109)	**0.0022**	20 (15–66)	64 (19–11224)	**0.0021**	17 (11–37)	157 (41–12522)	**0.0001**	19 (11–34)	71 (23–258)	**0.0009**
IL-7	88 (14–281)	386 (317–651)	**0.0001**	42 (16–191)	62 (17–325)	0.1555	18 (16–95)	30 (21–120)	0.1299	17 (14–222)	28 (23–102)	0.4206
IL-10	116 (91–720)	3712 (2689–4915)	**<0.0001**	148 (65–590)	1241 (392–3505)	**<0.0001**	75 (38–439)	1150 (269–3586)	**<0.0001**	111 (77–146)	1627 (265–3574)	**<0.0001**
IL-13	39 (30–72)	271 (116–910)	**<0.0001**	36 (16–834)	457 (52–14920)	**<0.0001**	32 (17–78)	1141 (179–13325)	**<0.0001**	27 (19–306)	553 (295–2632)	**<0.0001**
IL-17	301 (201–375)	454 (304 –803)	**0.0043**	391 (299–636)	774 (402–1918)	**0.0022**	382 (166–585)	803 (292–1426)	**0.0021**	227 (117–431)	522 (411–613)	**0.0023**
IFN-γ	61 (38–116)	246 (129–418)	**0.0006**	113 (50–296)	328 (159–8763)	**<0.0001**	57 (26–156)	633 (180–1958)	**<0.0001**	30 (15–83)	723 (274–1203)	**<0.0001**
TNF-α	1453 (212–13209)	6287 (760–23545)	**0.0004**	726 (146–19664)	4187 (1488–21345)	**0.0005**	8937 (170–20523)	14737 (1516–22729)	**0.0009**	980 (38–10521)	4664 (3168–17939)	**<0.0001**
G-CSF	143 (63–472)	1553 (1209–3636)	**<0.0001**	248 (118–2163)	1382 (359–4611)	**<0.0001**	90 (43–155)	574 (146–1507)	**0.0001**	51 (28–138)	818 (188–2338)	**<0.0001**
IL-1β	8441 (919–10423)	17268 (10218–20270)	**0.0003**	5354 (1563–14489)	13169 (7553–21871)	**0.0004**	2612 (220–7652)	12343 (3258–16967)	**<0.0001**	1498 (1138–5014)	10337 (4940–20003)	**<0.0001**
IL-4	90 (68–160)	284 (165–319)	**<0.0001**	139 (77–286)	288 (165–963)	**0.0006**	84 (61–190)	269 (169–1526)	**0.0001**	69 (31–114)	345 (246–443)	**0.0002**
IL-6	4950 (578–13495)	24495 (13348–26687)	**<0.0001**	7587 (2341–23094)	22686 (14780–25049)	**<0.0001**	3648 (227–13256)	19761 (13950–24652)	**<0.0001**	1198 (53–8694)	22609 (13532–24498)	**<0.0001**
GM-CSF	387 (190–432)	723 (409–783)	**<0.0001**	366 (233–943)	832 (403–1470)	**0.0004**	278 (113–679)	570 (389–1988)	**0.0008**	132 (48–455)	415 (323–628)	0.006

[a]The only cytokines with levels more elevated in the PHA-tube than in the unstimulated tube (background) are represented
[b]Results are given as median, (range) concentrations, pg/ml
p-values comparing background with PHA values were determined using the Mann–Whitney test
p-values below 0.0038 thresholds (corresponding to Bonferroni adjustment for 13 tests) are highlighted in bold

IL-1 beta and IL-6 are multifunctional pro-inflammatory cytokines also implicated in supporting Th2 and/or Th17 responses[39]. A drop during infancy and/or a more progressive decrease of TLR- and NLR-mediated IL-1 beta and IL-6 production have been reported by some thought not all[20]. In this study, a trend towards a slow decrease in IL-1 beta production with aging was observed while no change in IL-6 levels from birth to adulthood was noticed.

Our study also substantiates the aspect of a physiological deficiency for IL-17 production in neonates[14,16]. In addition, our study is the first to document a progressive increase in IL-17 secretion capacity that evolves in parallel with Th1 and Th2 maturation over time. As naive T-cells do not generally secrete Th1, Th2, and Th17 cytokines, the progressive increase in the expression of these cytokines might reflect antigen-driven T-cell differentiation as the child encounters a growing number of antigens. According to the current literature, specific intrinsic functional programs involving epigenetic modifications and variations in suppression factors are likely to also contribute to the maturation process of these adaptive cytokines.

In contrast, IL-10 production was higher at birth compared to later age and dropped to adult levels yet in infants. Rainsford et al. compared IL-10 production by isolated naive T-cells and demonstrated that cord blood $CD4^{+}CD45^{+}$ T-cells produce greater amounts of IL-10 upon T-cell-specific stimulation than their adult counterparts[25]. These authors however did not address the maturation of IL-10 production after birth. Discrepant with our results, Dirix et al. analyzed cytokine production by peripheral blood mononuclear cells (PBMCs) from premature and full-term neonates and reported that PHA-induced levels of IL-10 were less in neonates than in adults. Further analysis of cytokine development from birth to 16 months in the premature group revealed that IL-10 levels remain stable over time[5]. Although, the reasons for the discrepancies between Dirix's study and ours are not clear, we note that our study employed whole blood instead of PBMC. Neonatal mononuclear cells suspended in neonatal plasma show increase production of IL-10 compared to cells suspended in adult plasma[40]. Distinct study setting may thus, at least in part, account for the discrepancies between Dirix study and ours. IL-10 is a potent immunosuppressive cytokine that has a broad range of cellular sources including different T-cell subsets and B cells of the adaptive compartment and also various cell population of the innate immune system. The significant fall in IL-10 secretion level we observed in this study that used PHA a potent T-cell activator is reminiscent with the rapid decrease over time in IL-10 expression by monocytes after TLR stimulation[20]. Estimates of the age at which IL-10 production by innate cells following TLR stimulation is stabilized to adult levels vary from the second month to the second year in previous studies[20]. It is worth noting that IL-10 drop off to adult levels appears to occur more rapidly in our model using PHA for stimulation (within the first weeks of life). This apparent discrepancy invites to further compare the kinetics over time of IL-10 expression by innate and adaptive cells.

In addition to considering cytokines independently, the calculation of cytokine ratios may be an appropriate additional approach to interpret the functions of immune system. Using this approach, this study identified a previously unappreciated change from an inverse to a direct correlation between IL-10 and Th1, Th2, and Th17 cytokine levels soon after birth. Physiologically, a bias toward IL-10 over Th1, Th2, and Th17 secretion in fetuses could be critical for maintaining intra-uterine feto-maternal tolerance and preventing overwhelming inflammation during labor. After birth, the immune system must suddenly deal with a myriad of antigens, some potentially pathogenic. Immune responses must be effective in the fight against pathogens but at the same time must not have adverse effects on the host. Under these conditions, it seems reasonable to hypothesize that a positive correlation between the secretion of Th1/Th2/Th17 and IL-10 is optimal to ensure this

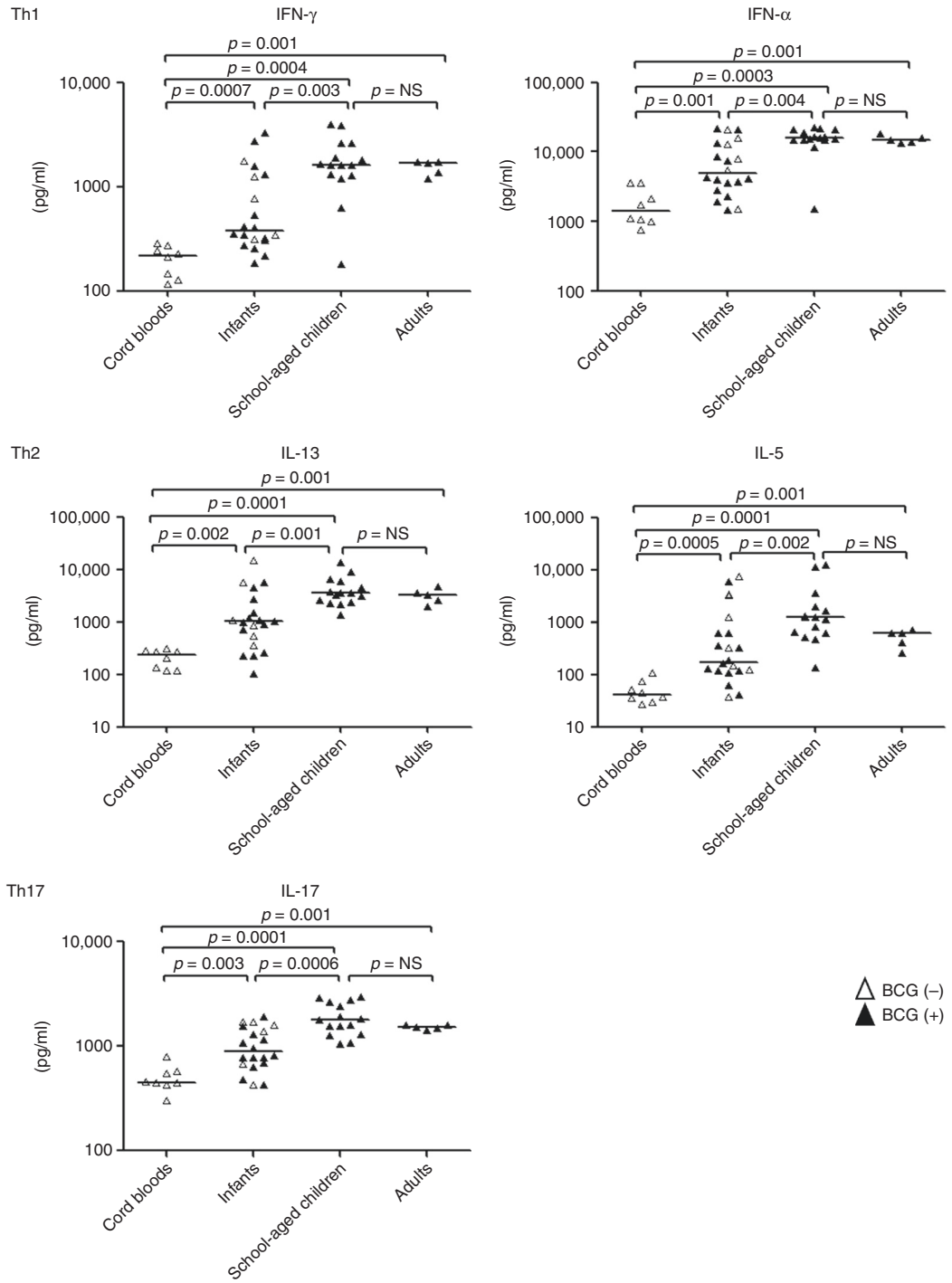

Fig. 1 Changes in the levels of IFN-γ, TNF-α, IL-13, IL-5 and IL-17 cytokine production in PHA-stimulated whole blood according to age. Results are expressed as individual values (pg/ml) in PHA-stimulated whole blood from BCG-vaccinated (black triangle), and BCG-not vaccinated (white triangle) individuals. Median values are symbolized by a horizontal bar for each age group. *P*-values (Mann–Whitney *U* test) are considered significant when <0.05

balance at that time. It is well admitted that the neonatal immune response is rapidly modulated at birth through encounters with environmental antigens, immune activators and biological response modifiers, including those present in colonizing microorganisms and dietary substances. The main factors responsible for the change from a negative to a positive correlation between IL-10 and Th1, Th2, and Th17 cytokines soon after birth remain to be elucidated.

Another point that has not been reported so far is the increased secretion capacity of IL-7, G-CSF, and IL-2 expression from high levels at birth that subsequently decrease during the first year of life. This observation suggests an important role for these cytokines at early stages of immune development. IL-7 provides trophic and proliferative signals for lymphopoiesis. The increased values of IL-7 coincide with the well-known increased values of lymphocyte numbers during the first months of life. High IL-7

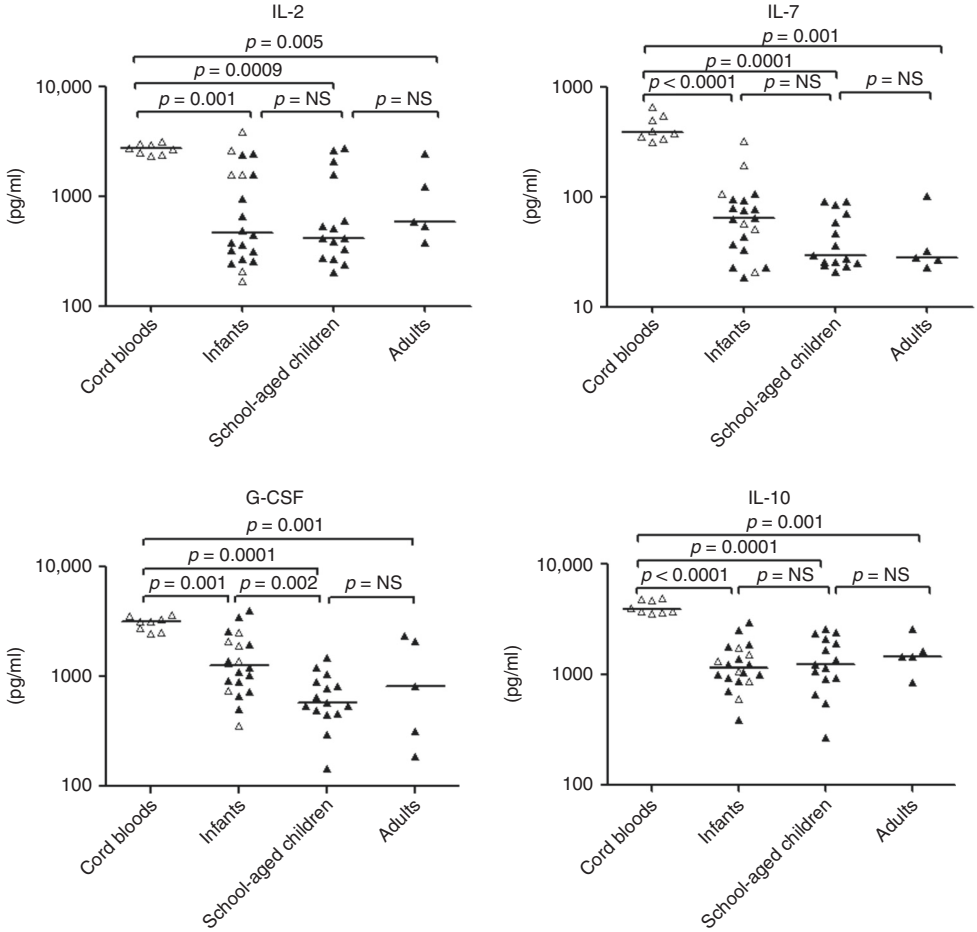

Fig. 2 Changes in the levels of IL-2, IL-7, G-CSF and IL-10 cytokine production in PHA-stimulated whole blood according to age. Results are expressed as individual values (pg/ml) in PHA-stimulated whole blood from BCG-vaccinated (black triangle), and BCG-not vaccinated (white triangle) individuals. Median values are symbolized by a horizontal bar for each age group. *P*-values (Mann–Whitney *U* test) are considered significant when <0.05. Lyophilized BCG vaccines (single intradermal injection) used the Moreau strain in infants and the Danish strain in older populations (Sanofi-Pasteur, France)

secretion capacity may contributes to this proliferation. G-CSF promotes the release of neutrophil progenitors from the bone marrow and increases neutrophil survival in the circulation. High levels of G-CSF, associated with the well-known increase in rates of neutrophils in the early newborn may contribute to improving granulopoiesis and/or optimal recruitment of neutrophils involved in the first line of defense against pathogens, before the establishment of an effective adaptive immune response. IL-2 regulates Treg homeostasis and function. Increased IL-2 secretion capacity until birth may be important in maintaining feto-maternal tolerance through Treg expansion. At birth, the newborn is confronted with large numbers of antigens. IL-2 is crucial in antigen-specific T-cell activation and differentiation. Whether reduced IL-2 responsiveness in infants might protect the developing child from potentially dangerous effector responses is an open question.

This study has strengths and limitations. Whole blood is an attractive minimally perturbed model system that assesses immune function in biologic fluid (plasma) containing immunomodulatory components. A whole blood stimulation assay using PHA, a polyclonal activator, however limits conclusions on cell-specific cytokine responses as many of the cytokines measured in our study could be produced by many cell types contained in whole blood. Although it is possible that PHA-reactive cells in whole blood may be of both innate and adaptive origin, a prominent expression of cytokines from the adaptive compartment in our model is likely for two reasons. (i) PHA is known to activate more actively adaptive immune cells than innate immune cells. (ii) As so, PHA failed to induce the innate IL-12 cytokine (this report), whereas LPS, a TLR agonist that activates innate immune cells did induce IL-12 in our hands (not shown). Finally, despite the small size of the cord blood group, the results reported here are sufficiently reliable to support differences in cytokine secretion capacity between neonates (cord blood) and infants.

In conclusion, under our culture conditions, a bias towards Th2-, Th17-cell polarizing cytokines in early life was not observed. Yet, high IL-10 expression over Th1, Th2, and Th17 expression at birth strengthens the belief that the fetal immune system is functionally programmed to promote tolerance. A dramatic drop in IL-10 expression and the occurrence of a positive correlation between IL-10, Th1, Th2, and Th17 expression soon after birth highlights the rapid adaptation of the early life immune system to fight infection and at the same time, to avoid an overwhelming inflammatory reaction. Further studies are required to identify the mechanisms that drive changes in IL-10 secretion capacity early in life.

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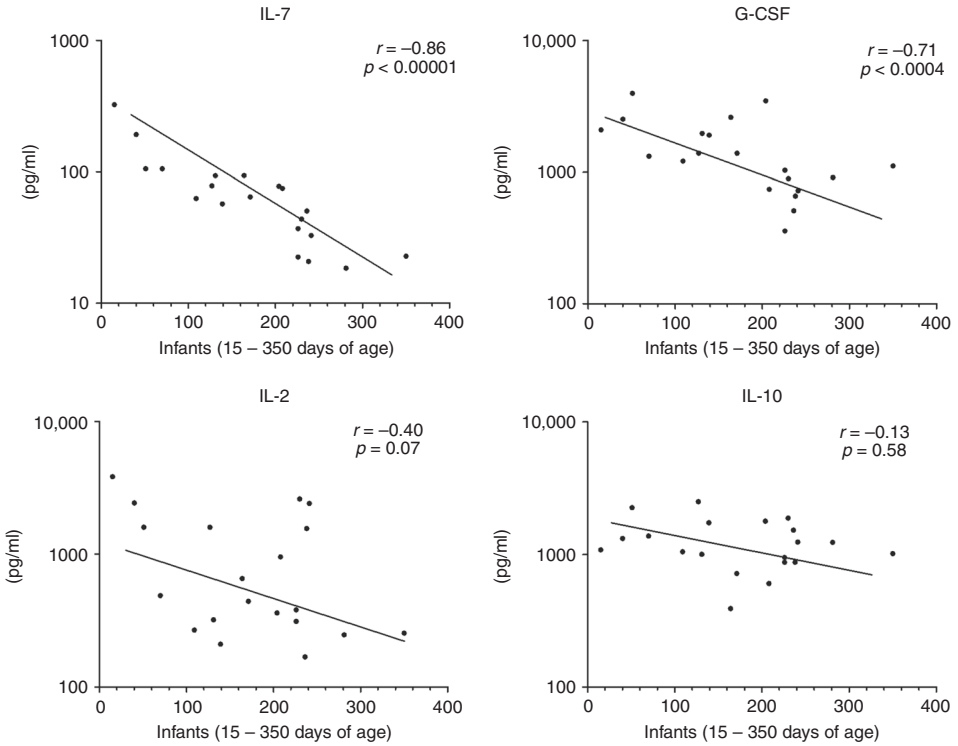

Fig. 3 Changes in the levels of cytokine production in PHA-stimulated whole blood from 15 days to 11 months of age. Data are expressed as individual values according to age. The non-parametric correlation between age and cytokine levels using Spearman's rho test (r) are given in the graph

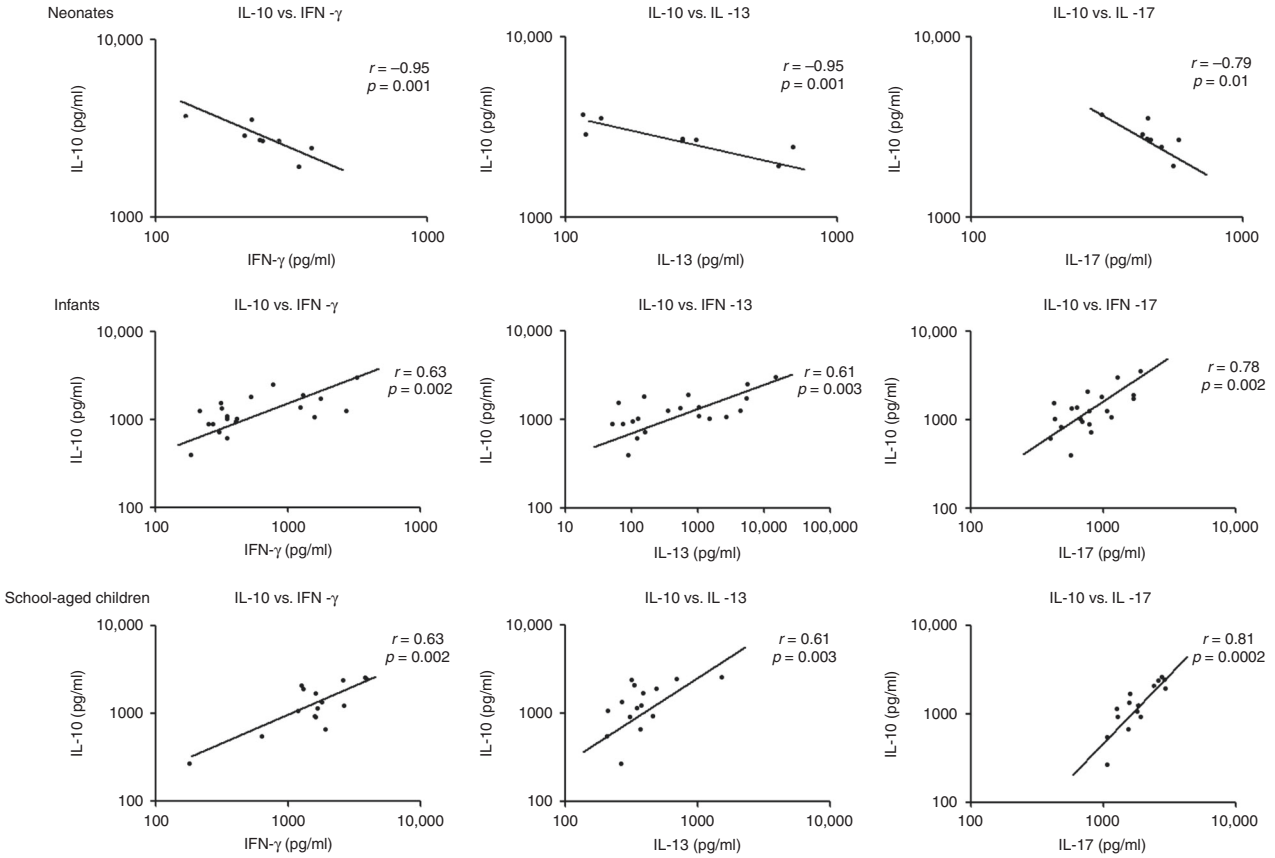

Fig. 4 Changes in the balance between the levels of Th1, Th2, Th17 cytokines, and of IL-10 in PHA-stimulated whole blood from neonates (cord blood), infants, and school-aged children. Results are expressed as individual values (pg/ml) according to age. Correlation (r) was analyzed using the Spearman's correlation test

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ADDITIONAL INFORMATION

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