

Letter to the Editor

Prelabor cesarean section bypasses natural immune cell maturation

To the Editor:

The perinatal milieu is believed to program the immune system of the child and play a role in the later incidence of immune-mediated diseases.¹ Although pregnancy seems to be a period controlled by anti-inflammatory hormones to prevent fetal rejection,² levels of maternal hormones are turned upside down at the process of giving birth and labor may be initiated by proinflammatory changes.³ These dramatic shifts during the last weeks of pregnancy may affect not only the mother but also the child.

Cesarean section (C-section) has been associated with increased risk of immune-mediated diseases⁴ during childhood, suggesting a delivery-mode-to-disease linkage that may involve the immune system.⁵ The prevalence of immune-mediated diseases has increased during the last few decades³ with a parallel increase in the rate of C-sections.⁶ However, the causal path from C-section to immune disease is not clear.⁷ Previous studies have demonstrated differences in microbial colonization patterns between neonates after C-section versus natural birth,⁸ but lung development, initiation of breast-feeding, and the degree of stress experienced by the child during birth may also be affected by the mode of delivery.

We hypothesized that initiation of labor may interfere with the natural maturation of the fetal immune system, including

circulating immune cells. We performed an extensive profiling of 23 innate and adaptive immune cell subsets. The immune subsets were compared against mode of delivery and gestational age in newborns enrolled in the Copenhagen Prospective Studies on Asthma in Childhood 2010 birth cohort.⁹

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and approved by the Ethics Committee for Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (j.nr. 2008-41-2599). The 91 study participants were all singletons delivered at term (week 37-42). Prelabor and in-labor C-section were defined as delivery by C-section before or after initiation of birth, respectively. General baseline characteristics were comparable between children delivered by C-section and those born naturally (see Table E1 in this article's Online Repository at www.jacionline.org). Leukocyte subpopulations, classified on the basis of expression of defined surface markers, were analyzed in cord blood by flow cytometry using the antibodies listed in Table E2 and the gating strategy shown in Fig E1 (see a detailed methods description in this article's Online Repository at www.jacionline.org). Each cell population was expressed as relative frequencies of CD45⁺ leukocytes (see Table E3 in this article's Online Repository at www.jacionline.org).

Univariate statistical *t* tests identified 7 individual immune cell subsets that were significantly different in neonates delivered by C-section, with increased relative frequencies of blood dendritic

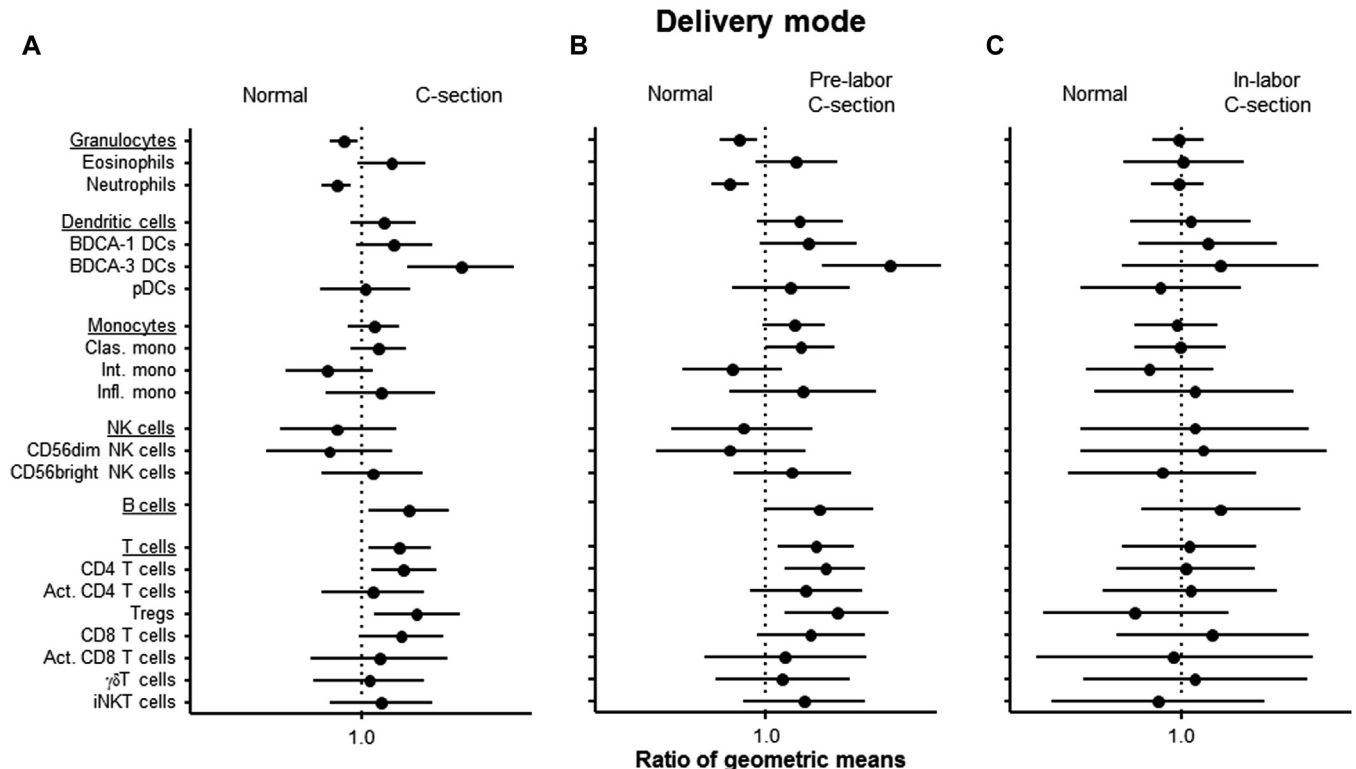


FIG 1. Cord blood immune cell frequencies in newborns delivered by C-section as compared with newborns delivered by natural birth. Data represent the ratio of geometric means with 95% CIs of immune cell subsets in cord blood of children born by C-section (A, *n* = 20), prelabor C-section (B, *n* = 14), or in-labor C-section (C, *n* = 6) as compared with children born by natural birth (*n* = 71). Act., Activated; clas., classical; infl., inflammatory; iNKT, invariant NKT; int., intermediate; pDC, plasmacytoid DC; Tregs, regulatory T cells.

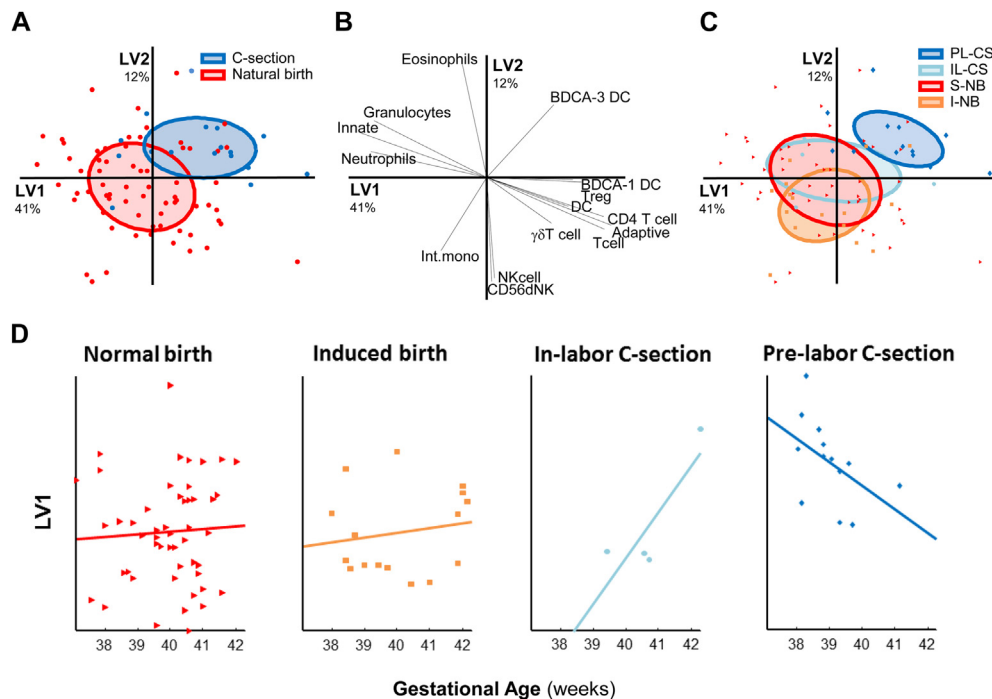


FIG 2. Cord blood immune cell profile and delivery mode in relation to gestational age. **A** and **B**, PLSDA model of cord blood immune cell distributions separating C-section and natural-born neonates. Fig 2, A, In the score plot, each dot represents the composition of immune cell subsets of a single child. Dots are colored by delivery mode, and shaded areas represent ± 1 SD spatially. Fig 2, B, The associated loading plot depicts the profile of cord blood immune cells separating infants born by C-section and natural birth. **C**, The score plot from the PLSDA model in Fig 2, A, labeled and colored by prelabor C-section (PL-CS), in-labor C-section (IL-CS), spontaneous natural birth (S-NB), and induced natural birth (I-NB). **D**, Correlation of maternal gestational age with the immune cell profile represented by LV1 for natural birth ($n = 51$), induced birth ($n = 16$), IL-CS ($n = 4$) and PL-CS ($n = 13$). *Int.mono*, Intermediate monocytes; *Treg*, regulatory T cells.

cell antigen-3 dendritic cells (BDCA-3 DCs) ($P < .001$), regulatory T cells ($P = .01$), CD4 T cells ($P = .01$), T cells ($P = .02$), and B cells ($P = .03$) and reduced frequencies of neutrophils ($P < .001$) and granulocytes ($P = .01$), than in neonates born by natural delivery (Fig 1, A). Analyzing prelabor and in-labor C-section to natural birth showed that prelabor C-section was responsible for the C-section-associated difference, with altered relative frequencies of the same immune subsets except for B cells (Fig 1, B; see Table E4 in this article's Online Repository at www.jacionline.org). No difference in individual immune cell frequencies was observed in children delivered by in-labor C-section as opposed to natural birth (Fig 1, C; see Table E4).

Because the analyzed immune subsets were highly correlated and nested within each other, we used partial least squares discriminant analysis (PLSDA) to reduce the number of variables from 23 immune cells to fewer discriminatory latent variables (LVs). The resulting PLSDA model divided the newborns with respect to the mode of delivery (Fig 2, A) on the basis of a specific pattern of immune cell frequencies (Fig 2, B) with a cross-validated sensitivity and specificity of 0.76 ($P = .003$). The model had 2 LVs that explained 53% (41% and 12%, respectively) of the total variation within the immune cell subsets (Fig 2, A). Specifically, the PLSDA model identified elevated levels of BDCA-3 DCs in children born by C-section, along with a pattern of increased frequencies of eosinophils, BDCA-1 DCs, DCs, regulatory T cells, CD4 T cells, T cells, adaptive immune cells, and $\gamma\delta$ T cells and reduced levels of intermediate

monocytes, neutrophils, granulocytes, innate immune cells, CD56dim natural killer cells, and natural killer cells, than in children born naturally (Fig 2, B). We further refined the analysis by mode of C-section—prelabor and in-labor C-section—and by method of natural birth—induced and spontaneous natural birth—and the score plot was labeled and colored according to the 4 modes of delivery (Fig 2, C). Based on the distribution of cord blood immune cells, it was apparent that the separation between newborns born by C-section versus natural birth was driven by a different immune cell distribution in children delivered by prelabor C-section (Fig 2, C). We identified a correlation between gestational age and the immune cell distribution within prelabor C-section-delivered newborns described by LV1 ($r = -0.63$; $P = .02$; Fig 2, D). That is, the pattern of immune cells of prelabor C-section newborns with lower gestational age was unique, whereas immune cell distributions within prelabor C-section children with higher gestational age and within children delivered by natural birth were more alike. Gestational age did not influence the distribution of immune cells in children after spontaneous natural birth (Fig 2, D). No direct significant association was observed between gestational age and cord blood immune cell frequencies (data not shown).

Collectively, the profile of circulating innate and adaptive immune cells was observed to differ between neonates delivered by prelabor C-section and natural birth. To our knowledge, this is the first study to identify gestational age to be inversely correlated to the C-section-associated immune cell distribution within

prelabor C-section–delivered newborns. This gestational age–associated immune cell pattern implies that factors relating to pregnancy affect the fetal immune cell profile in a gradual manner during the final weeks of pregnancy, and this suggests the occurrence of immune programming alongside the onset of labor in term children. The link between C-section and increased risk of immune-mediated diseases is generally thought to be due to different bacterial colonization patterns dependent on the route of delivery.^{4,7} On the basis of our results, we propose that absence of labor-related *in utero* immune programming in prelabor C-section–born children may be a contributing factor to the reported association between C-section and immune-mediated diseases.⁴ Further understanding of these processes may help to understand and prevent unwanted effects of C-section in relation to disease programming of the child.

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Anna Hammerich Thysen, PhD^{a,b}

Jeppé Madura Larsen, PhD^b

Morten Arendt Rasmussen, PhD^{a,c}

Jakob Stokholm, PhD^a

Klaus Bønnelykke, PhD^a

Hans Bisgaard, DMSc^d

Susanne Brix, PhD^b

From ^aCopenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark; ^bthe Department of Systems Biology, Center for Biological Sequence Analysis, Technical University of Denmark, Kgs. Lyngby, Denmark; and ^cSpectroscopy and Chemometrics, Department of Food Science, University of Copenhagen, Copenhagen, Denmark. E-mail: bigaard@copsac.com.

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METHODS

Study cohort

Children participated in the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort, an ongoing unselected clinical prospective birth cohort of 700 children.^{E1} The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and approved by the Ethics Committee for Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (j.nr. 2008-41-2599). Both parents gave their informed consent before enrollment of the children.

The study participants were all singletons delivered at term (week 37-42) in hospitals in Zealand, Denmark. Gestational age was determined on the basis of ultrasonography, except for 1 child for whom information on last menstrual period was used. The distribution of modes of delivery was natural birth ($n = 71$) and C-section ($n = 20$). These were subdivided into induced natural birth ($n = 17$), spontaneous natural birth ($n = 54$), prelabor C-section ($n = 14$), or in-labor C-section ($n = 6$), respectively. Prelabor and in-labor C-section were defined as delivery by C-section before or after initiation of birth, respectively.

Cord blood was sampled by midwives into a citrate phosphate dextrose collection bag (catalog 791-01U, Pall, NY) by needle puncture of the umbilical vein either before or after delivery of the placenta.

Baseline

Baseline information on atopic heredity, birth parameters, prenatal exposures, and sociodemographic characteristics was recorded by trained staff during scheduled visits at COPSAC clinics at gestational week 24 and 36 and 1 week postpartum, and further supported by midwife's notes and registry diagnoses.

Flow cytometry of immune phenotypes

Leukocyte subpopulations were analyzed in cord blood within 72 hours of birth using a lyse-no-wash procedure. In prestudy optimizations, no effect of cord blood storage time was observed on the distribution of immune cell subsets. The procedure was as follows: 50 μ L of cord blood was added to 5 tubes, each containing a pretitrated 2- to 7-color antibody mixture (Table E2). After a 30-minute incubation period, erythrocytes were lysed in VersaLyse (Beckman Coulter, Brea, Calif). For absolute cell counts, 100 μ L of Flow-Count beads (Beckman Coulter) was added to one of the tubes. Approximately 1 mL of cell suspension was collected on a BD FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ) with fixed speed and a stopping time of 10 minutes. Leukocyte subpopulations were classified on the basis of the expression of defined surface markers as follows: leukocytes (CD45⁺), granulocytes (CD45⁺SSC^{int/high}), neutrophils (CD45⁺CD16⁺SSC^{int/high}), eosinophils (CD45⁺CD16⁺SSC^{high}), B cells (CD45⁺CD19⁺), T cells (CD3⁺), CD4 T cells ($\alpha\beta$ TCR⁺CD4⁺), CD8 T cells ($\alpha\beta$ TCR⁺CD8⁺), activated CD4 T cells ($\alpha\beta$ TCR⁺CD4⁺CD25⁺CD127^{high/+}), activated CD8 T cells ($\alpha\beta$ TCR⁺CD8⁺CD25⁺CD127^{high/+}), regulatory T cells ($\alpha\beta$ TCR⁺CD4⁺CD25⁺CD127^{low/-}), $\gamma\delta$ T cells ($\gamma\delta$ TCR⁺), invariant NKT cells (CD3⁺V α 24J α 18⁺), classical monocytes (CD3⁺CD19⁺CD56⁺CD14^{+/high}CD16⁻), intermediate monocytes (CD3⁺CD19⁺CD56⁺CD14^{+/high}CD16⁺), inflammatory monocytes (CD3⁺CD19⁺CD56⁺CD14^{int/-}CD16⁺), BDCA-1 DCs (CD3⁺CD19⁺CD56⁺CD14⁺CD16⁺BDCA-1⁺), BDCA-3 DCs (CD3⁺CD19⁺CD56⁺CD14⁺CD16⁺CD1c⁺CD303⁺BDCA-3^{high}), plasmacytoid

DCs (CD3⁺CD19⁺CD56⁺CD14⁺BDCA-2⁺), natural killer (NK) cells (CD3⁺CD56⁺), CD56dim NK cells (CD3⁺CD56^{dim}), CD56bright NK cells (CD3⁺CD56^{bright}), as well as overall numbers of monocytes, DCs, innate cells, and adaptive cells. Gating was finalized in FlowJo v. 7.6.5 (TreeStar, Ashland, Ore), and based on a predefined and unbiased gating strategy (Fig E1). Gated events were normalized by overlap of corresponding CD3⁺, CD4⁺, or CD8⁺ subsets between tubes.

Data analysis and statistics

Pearson χ^2 test or unpaired student t test was used to test for simple associations between baseline characteristics.

Cell populations were expressed as relative frequencies of CD45⁺ leukocytes. Our primary variable was C-section, with natural birth as reference group. Season of birth and/or time between birth and flow-cytometry analysis were tested as covariates. For each cell population, linear models (t tests) were used to study the inference with C-section, both adjusted and unadjusted. Both frequency and count data were base 10 log-transformed to obtain approximately normal distributed residuals. Estimates were expressed as relative differences of means, which after transformation back to the original scale became the ratio of geometric means. Univariate statistical inference was reported as uncorrected P values and supported by $\alpha = 0.05$ significance thresholds based on Bonferroni and the false-discovery rate by Benjamini-Hochberg.

PLSDA was used as a data-driven method to extract discriminatory latent variables.^{E2} These latent variables were derived to discriminate children born by C-section from children born by natural birth depending on the systemic variation between cord blood immune cell frequencies. Cell population to cell population correlation structure was visualized in the loading plot of the first 2 latent variables, and the corresponding score plot differentiated newborns according to birth method. By evaluation of the "Variables Important for Prediction criterion," immune cell populations that did not contribute to the segregation of delivery modes were excluded. These were plasmacytoid DCs, classical monocytes, inflammatory monocytes, CD56bright NK cells, B cells, CD8 T cells, and invariant NKT cells. The PLSDA model was 10-fold cross-validated to select the optimal number of components. To ascertain that the original model was not obtained by coincidence, inference of the multivariate model was assessed by random permutation testing of the cross-validated area under the receiver operator curve with 10,000 permutations.

Pearson product-moment correlation coefficient was used to compute associations between LVs and individual immune cells to gestational age, stratified for each mode of delivery.

Data were handled in RStudio v. 0.97.320 (RStudio, Boston, Mass) and MATLAB R2012b v. 7.9.0.529 (Mathworks, Natick, Mass), with PLS toolbox v. 6.5.1 for model estimation. Visualization and permutation testing were performed with *in-house* algorithms.

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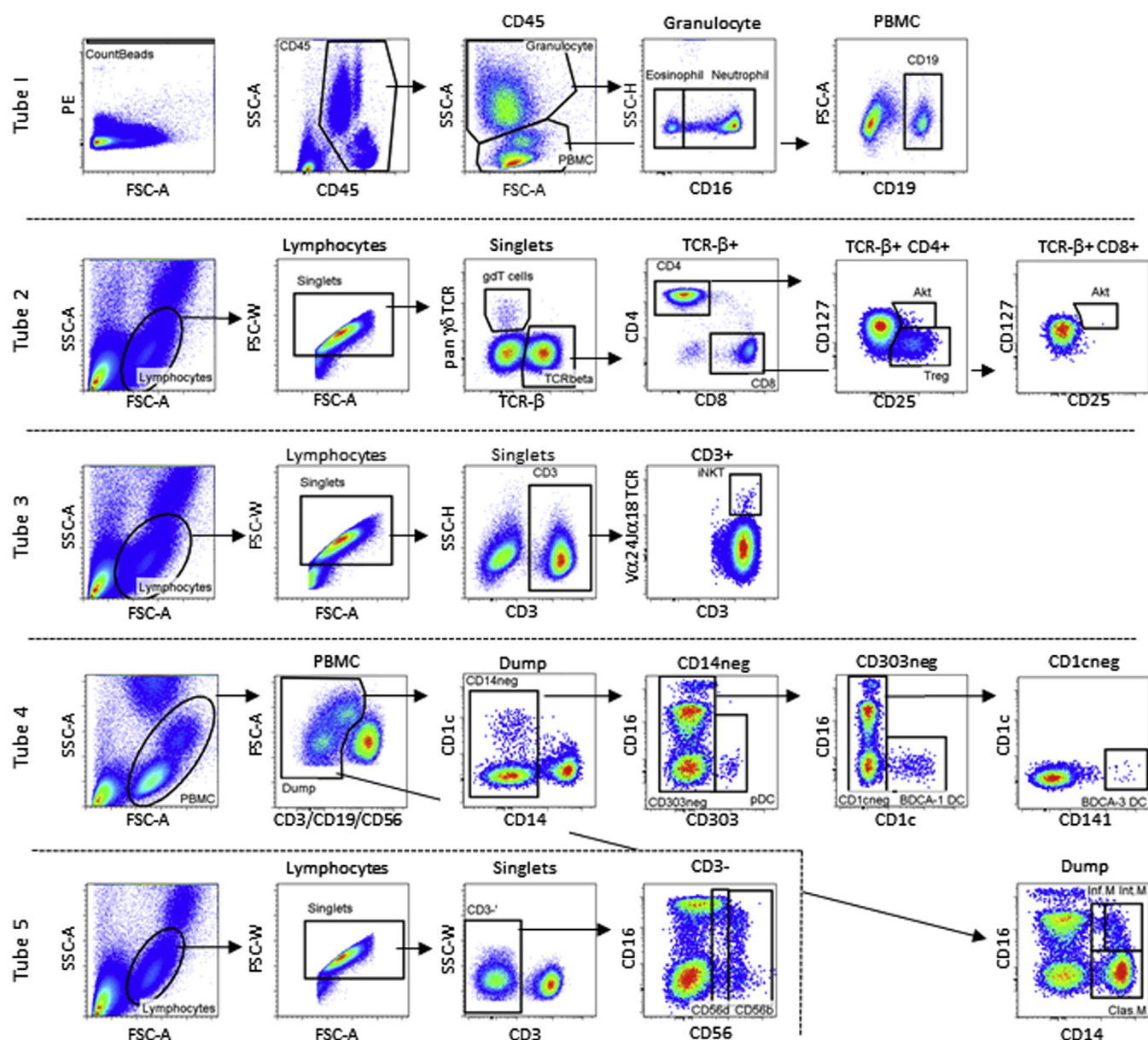


FIG E1. Gating strategy. Cord blood immune cell phenotypes are labeled in 5 separate tubes. Each tube is initially gated on forward-side scatter properties and singlets are selected on the basis of area versus height parameters of forward scatter (FSC). Flow-count beads, granulocyte subsets, and B cells are selected in tube 1. $\gamma\delta$ T cells, $\alpha\beta$ T cells, CD4 T cells, CD8 T cells, and regulatory T (Treg) cells are quantified within tube 2 and invariant NKT cells in tube 3. Subsets of DCs and monocytes are gated in tube 4, while NK cells are detected in tube 5. PE, Phycoerythrin; SSC, side scatter.

TABLE E1. Baseline demographic characteristics of children born by C-section and natural birth

Characteristic	Study cohort	Natural birth	C-section		Prelabor C-section		In-labor C-section	
			P value		P value		P value	
N	91	71	20		14		6	
Atopic heredity*, n (%)								
Maternal asthma	27 (30)	20 (28)	7 (35)	.75†	6 (43)	.44†	1 (17)	.90†
Paternal asthma	20 (22)	15 (21)	5 (25)	.98†	2 (14)	.81†	3 (50)	.28†
Maternal rhinitis	25 (27)	19 (27)	6 (30)	.99†	4 (29)	.99†	2 (33)	.99†
Paternal rhinitis	23 (26)	18 (25)	5 (25)	1.00†	3 (21)	.98†	2 (33)	.99†
Maternal dermatitis	23 (25)	19 (27)	4 (20)	.75†	3 (21)	.93†	1 (17)	.95†
Paternal dermatitis	15 (17)	11 (15)	4 (20)	.93†	3 (21)	.91†	1 (17)	.99†
Birth								
Apgar score of >9 at 5 min, n (%)	86 (95)	68 (96)	18 (90)	.66†	13 (93)	.99†	5 (83)	.72†
Birth length (cm), mean ± SD	52 ± 2.2	52.1 ± 2.2	52.4 ± 1.9	.55‡	51.9 ± 1.7	.74‡	53.7 ± 1.9	.10‡
Birth weight (kg), mean ± SD	3.6 ± 0.5	3.6 ± 0.5	3.7 ± 0.5	.34‡	3.6 ± 0.4	.99‡	4.0 ± 0.6	.07‡
Gestational age (wk), mean ± SD	40.0 ± 1.3	40.0 ± 1.2	39.6 ± 1.3	.16‡	39.0 ± 0.8	.003‡	41.0 ± 1.1	.06‡
Child								
Sex: male, n (%)	52 (57)	43 (61)	45 (9)	.32†	36 (5)	.16†	67 (4)	.99†
Prenatal exposures								
Alcohol in pregnancy§, n (%)	4 (4)	2 (3)	2 (10)	.44†	0 (0)	.99†	2 (33)	.03†
Antibiotics in pregnancy, n (%)	33 (36)	25 (35)	8 (40)	.90†	8 (57)	.21†	0 (0)	.19†
Cat in household in pregnancy, n (%)	23 (26)	17 (24)	6 (30)	.82†	4 (29)	.99†	2 (33)	.99†
Dog in household in pregnancy, n (%)	22 (24)	15 (21)	7 (35)	.34†	6 (43)	.18†	1 (17)	.99†
Smoking in pregnancy, n (%)	9 (10)	7 (10)	2 (10)	1.00†	1 (7)	.99†	1 (17)	.99†
Sociodemographic characteristic								
Any older siblings, n (%)	51 (56)	37 (52)	14 (70)	.24†	12 (86)	.04†	2 (33)	.65†
Household income , n (%)	42 (46)	32 (45)	10 (50)	.89†	6 (43)	.36†	4 (67)	.51†
Mother's age at birth (y), mean ± SD	32.0 ± 4.3	31.7 ± 4.6	33.3 ± 4.7	.16‡	32.6 ± 3.9	.47‡	34.9 ± 6.3	.11‡
Mother's highest education¶, n (%)	27 (30)	19 (27)	8 (40)	.39†	5 (35)	.57†	3 (50)	.44†
Nulliparous mother, n (%)	45 (49)	37 (52)	8 (40)	.48†	2 (14)	.02†	6 (100)	.07†

Baseline comparisons between the C-section and natural birth. P values below significance level .5 are in boldface.

*Doctor-diagnosed history of disease.

†Pearson χ^2 test.

‡Unpaired Student *t* test.

§Above 1 unit per week.

||Above €110,000.

¶University graduate.

TABLE E2. Antibodies for flow-cytometry analysis of cord blood immune phenotypes

Immune cell subset	Antigen	Conjugate	Clone	Manufacturer
Tube 1	CD16	PerCP-Cy5.5	3G8	BioLegend*
	CD45	APC-Alexa Flour 750	J33	Beckman Coulter
	CD19	eFlour450	HIB19	EBioscience*
Tube 2	CD4	AmCyan	SK3	BD Biosciences
	αβTCR	FITC	IP26	eBioscience
	γδTCR	PE	IMMU510	Beckman Coulter
	CD25	PC5.5	B1.49.9	Beckman Coulter
	CD127	APC	eBioRDR5	eBioscience
Tube 3	CD4	eFlour450	SK3	eBioscience
	CD8	AmCyan	SK1	BD Biosciences
	CD1c	FITC	L161	eBioscience
	CD141	PE	1A4	BD Biosciences
	CD16	PerCP-Cy5.5	3G8	BioLegend
	CD14	PE-Cy7	61D3	eBioscience
	CD303	APC	AC144	Miltenyi Biotec01†
	CD3	eFlour450	UCHT1	eBioscience
	CD19	eFlour450	HIB19	eBioscience
	CD56	Pacific Blue	MEM-188	BioLegend
Tube 4	CD4	AmCyan	SK3	BD Biosciences
	CD161	APC	HP-3G10	BioLegend
	CD56	PE-Cy7	MEM-188	BioLegend
	CD3	APC-Cy7	UCHT1	eBioscience
	CD16	eFlour450	CB16	eBioscience
Tube 5	CD8	AmCyan	SK1	BD Biosciences
	TCR Vα24-Jα18	APC	6B11	eBioscience
	CD3	eFlour450	UCHT1	eBioscience

*San Diego, Calif.

†Bergish Gladbach, Germany.

TABLE E3. Baseline levels of cord blood immune cells by delivery mode

Immune cell subset	Percentage of above population*	Median (IQR)†			
		Natural birth	C-section	Prelabor C-section	In-labor C-section
N		71	20	14	6
Granulocytes	51	52.5 (46.6-57.7)	47.6 (39.3-52.3)	45.9 (38.7-48.0)	54.0 (49.6-58.07)
Eosinophils	11	5.17 (3.82-6.61)	6.25 (4.34-8.51)	6.25 (4.62-8.60)	5.57 (3.97-7.93)
Neutrophils	89	47.3 (41.5-52.5)	39.8 (33.8-45.5)	38.8 (33.1-40.1)	46.9 (43.4-51.2)
DCs	0.2	0.20 (0.15-0.25)	0.23 (0.17-0.30)	0.25 (0.20-0.31)	0.20 (0.16-0.27)
BDCA-1 DCs	66	0.12 (0.08-0.17)	0.18 (0.12-0.21)	0.18 (0.13-0.21)	0.15 (0.10-0.20)
BDCA-3 DCs	3	0.45 (0.31-0.62)‡	1.06 (0.69-1.28)‡	1.22 (0.74-1.30)‡	0.75 (0.47-1.02)‡
Plasmacytoid DCs	31	5.92 (3.87-8.62)‡	6.67 (4.02-7.44)‡	6.84 (4.90-8.43)‡	5.23 (3.70-6.80)‡
Monocytes	7	6.33 (5.27-7.69)	7.11 (5.45-7.60)	7.50 (6.67-8.22)	5.63 (5.44-6.62)
Classical monocytes	89	5.58 (4.60-6.77)	6.60 (4.95-6.86)	6.82 (6.17-7.33)	5.06 (4.91-6.16)
Intermediate monocytes	9	0.51 (0.34-0.83)	0.39 (0.30-0.51)	0.43 (0.29-0.49)	0.35 (0.33-0.53)
Inflammatory monocytes	2	0.12 (0.06-0.18)	0.13 (0.09-0.14)	0.13 (0.10-0.15)	0.10 (0.09-0.12)
NK cells	2	1.57 (1.03-2.49)	1.36 (0.81-1.95)	1.07 (0.76-1.95)	1.41 (1.36-1.65)
CD56dim NK cells	81	1.26 (0.79-2.04)	1.13 (0.59-1.39)	0.79 (0.56-1.39)	1.22 (1.11-1.48)
CD56bright NK cells	20	0.25 (0.17-0.41)	0.28 (0.21-0.32)	0.29 (0.21-0.32)	0.24 (0.21-0.27)
B cells	6	5.37 (4.11-6.74)	6.56 (5.13-8.56)	6.56 (5.22-8.69)	6.34 (4.63-7.91)
T cells	20	18.5 (13.9-23.8)	26.2 (16.2-28.2)	26.7 (21.9-28.2)	15.7 (15.1-20.4)
CD4 T cells	67	12.2 (9.68-16.3)	16.6 (11.1-21.4)	17.5 (13.3-22.4)	10.3 (9.39-13.7)
Activated CD4 T cells	1	0.15 (0.10-0.24)	0.18 (0.11-0.27)	0.21 (0.13-0.27)	0.13 (0.07-0.18)
Treg cells	6	0.76 (0.54-0.95)	1.05 (0.72-1.38)	1.35 (0.76-1.45)	0.71 (0.66-0.82)
CD8 T cell	20	3.55 (2.61-5.23)	3.98 (3.51-5.87)	4.53 (3.81-5.87)	3.61 (3.35-4.66)
Activated CD8 T cells	0.3	0.71 (0.42-1.32)‡	0.76 (0.48-1.45)‡	1.02 (0.60-1.45)‡	0.55 (0.46-0.96)‡
γδT cells	2	0.32 (0.17-0.44)	0.33 (0.22-0.52)	0.33 (0.23-0.52)	0.30 (0.21-0.43)
Invariant NKT cells	0.3	5.02 (3.81-8.19)‡	7.16 (3.81-9.60)‡	7.92 (5.47-9.60)‡	3.78 (3.35-6.31)‡

IQR, Interquartile range; Treg, regulatory T.

*Each cell population is expressed as a mean percentage of its immediate upper population.

†Individual cell subsets are given as the median percentage of CD45⁺ leukocytes and IQR.‡Multiplied by 10².

TABLE E4. Associations between modes of C-section and immune cell fractions in cord blood

Immune cell subset	C-section/natural birth		Prelabor C-section/natural birth		In-labor C-section/natural birth	
	Ratio of means (95% CI)*	P value	Ratio of means (95% CI)*	P value	Ratio of means (95% CI)*	P value
Granulocytes	0.89 (0.81-0.97)	.01	0.84 (0.74-0.94)	.004	1.02 (0.92-1.14)	.64
Eosinophils	1.21 (0.97-1.50)	.09	1.21 (0.93-1.59)	.16	1.21 (0.95-1.55)	.13
Neutrophils	0.85 (0.77-0.93)	.001	0.79 (0.70-0.89)	<.001	1.00 (0.90-1.11)	.95
DCs	1.15 (0.93-1.42)	.21	1.24 (0.94-1.64)	.12	1.02 (0.79-1.30)	.89
BDCA-1 DCs	1.23 (0.96-1.57)	.10	1.31 (0.96-1.80)	.09	1.05 (0.79-1.40)	.72
BDCA-3 DCs	1.90 (1.34-2.68)	<.001	2.23 (1.43-3.48)	<.001	1.17 (0.80-1.73)	.41
Plasmacytoid DCs	1.02 (0.76-1.36)	.92	1.17 (0.80-1.71)	.41	0.86 (0.61-1.21)	.39
Monocytes	1.08 (0.91-1.27)	.37	1.20 (0.98-1.46)	.07	0.87 (0.72-1.05)	.14
Classical monocytes	1.11 (0.93-1.33)	.23	1.25 (1.00-1.55)	.05	0.87 (0.71-1.08)	.20
Intermediate monocytes	0.80 (0.61-1.07)	.13	0.80 (0.58-1.11)	.18	0.87 (0.63-1.20)	.39
Inflammatory monocytes	1.13 (0.79-1.61)	.51	1.27 (0.79-2.03)	.31	0.66 (0.43-1.00)	.05
NK cells	0.85 (0.59-1.25)	.41	0.86 (0.54-1.35)	.50	0.78 (0.52-1.17)	.22
CD56dim NK cells	0.81 (0.54-1.22)	.31	0.79 (0.49-1.29)	.34	0.76 (0.49-1.18)	.22
CD56bright NK cells	1.07 (0.77-1.48)	.70	1.18 (0.81-1.73)	.38	0.86 (0.61-1.22)	.39
B cells	1.35 (1.04-1.76)	.03	1.41 (0.99-1.99)	.05	0.86 (0.62-1.18)	.35
T cells	1.27 (1.04-1.56)	.02	1.38 (1.08-1.77)	.01	0.92 (0.74-1.16)	.49
CD4 T cells	1.31 (1.06-1.62)	.01	1.47 (1.13-1.90)	.004	0.91 (0.72-1.15)	.41
Activated CD4 T cells	1.07 (0.77-1.49)	.70	1.29 (0.90-1.86)	.17	0.86 (0.60-1.22)	.39
Treg cells	1.42 (1.08-1.88)	.01	1.58 (1.13-2.20)	.008	0.94 (0.69-1.29)	.71
CD8 T cell	1.29 (0.98-1.69)	.07	1.33 (0.94-1.89)	.11	0.92 (0.67-1.26)	.59
Activated CD8 T cells	1.12 (0.72-1.74)	.63	1.13 (0.67-1.92)	.64	0.90 (0.55-1.48)	.68
γδT cells	1.05 (0.73-1.49)	.80	1.11 (0.72-1.71)	.63	0.95 (0.64-1.42)	.81
Invariant NKT cells	1.13 (0.81-1.58)	.48	1.28 (0.86-1.90)	.23	0.88 (0.62-1.26)	.48

After FDR adjustment for multiple comparisons, frequencies of BDCA-3 DCs, Treg cells, CD4 T cells, T cells, neutrophils, and granulocytes remained significantly different in prelabor C-section neonates.

FDR, False-discovery rate; Treg, regulatory T.

*Each cell subset is log-transformed before calculation of the univariate ratio of geometric means. The Bonferroni-corrected significance level was $P = .002$ and the FDR was $P = .01$ (both with error rate of 0.05). P values below the FDR-corrected significance level of $P = .010$ are in boldface.