Letter to the Editor

Prelabor cesarean section bypasses natural immune cell maturation

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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 diseases[4] 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 decades[3] 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

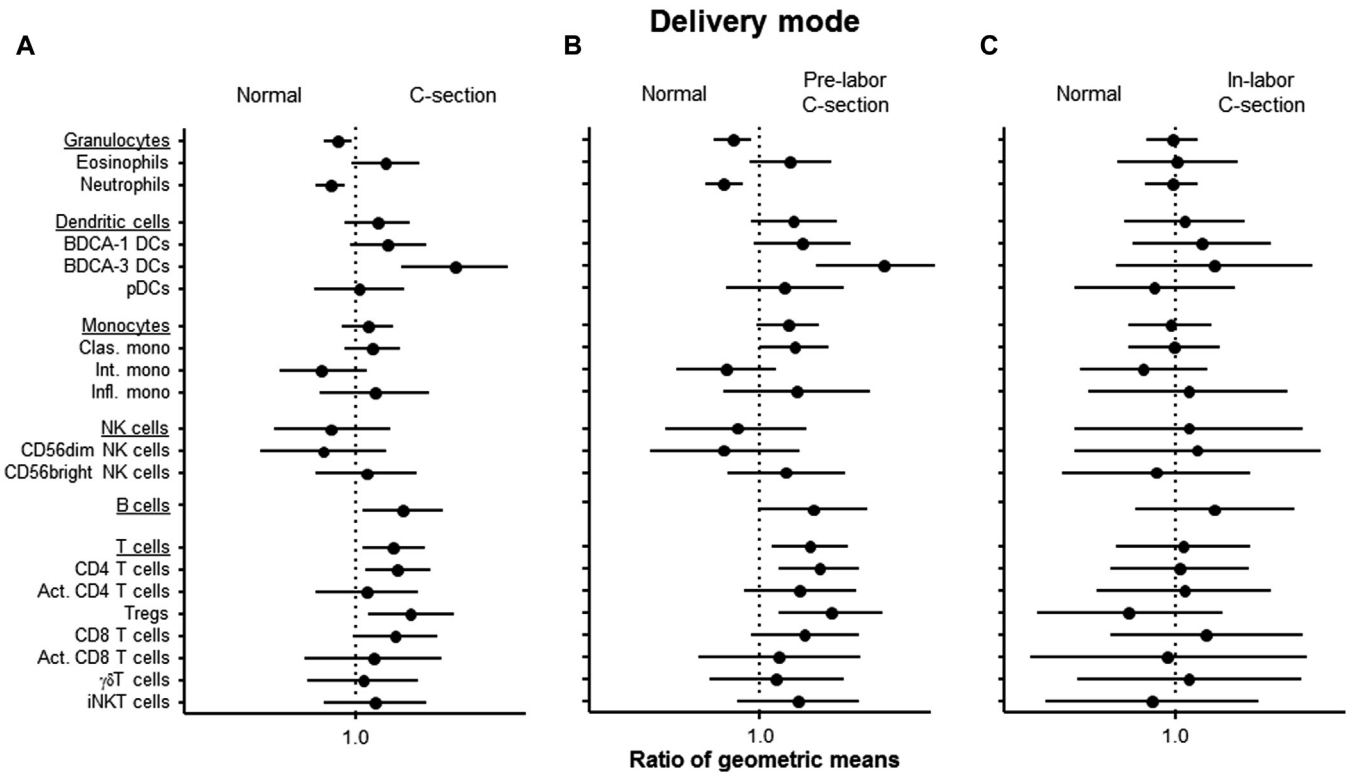

FIG 1. Cord blood immune cell frequencies in newborns delivered by C-section as compared with newborns delivered by natural birth. Data represent the ratio of geometric means with 95% CIs of immune cell subsets in cord blood of children born by C-section (**A,** n = 20), prelabor C-section (**B,** n = 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.

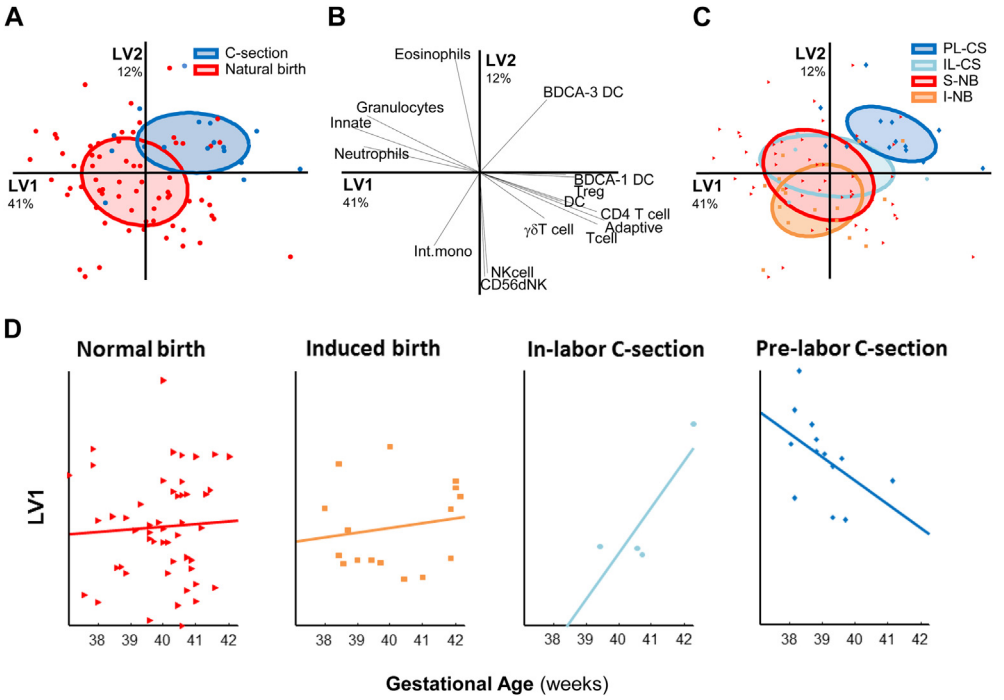

FIG 2. Cord blood immune cell profile and delivery mode in relation to gestational age. **A** and **B**, PLSDA model of cord blood immune cell distributions separating C-section and natural-born neonates. Fig 2, *A*, In the score plot, each dot represents the composition of immune cell subsets of a single 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 γδT 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