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Brief Communication

Compromised immune response in infants at risk for type 1 diabetes born by Caesarean Section

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ABSTRACT

Children born by Caesarean Section have a higher risk for type 1 diabetes. We aimed to investigate whether Caesarean Section leads to alterations of the immune response in children with familial risk for type 1 diabetes. We examined measures of innate and adaptive immune responses in 94 prospectively followed children, including 40 born by Caesarean Section. Proinflammatory serum cytokine concentrations were determined at age 6 months. As a measure of vaccine response, IgG1, IgG2, and IgG4 tetanus antibody titers and $CD4^+$ T cell proliferation against tetanus toxoid were quantified. Compared to infants born by vaginal delivery, infants born by Caesarean Section had lower concentrations of the cytokines IFN-ɣ ($p = 0.014$) and IL-8 ($p = 0.005$), and weaker $CD4^+$ T cell responses to tetanus measured in the first ($p = 0.007$) and second year ($p = 0.047$) of life. Overall, our findings provide evidence that the mode of delivery influences the immune status and responsiveness during childhood.

1. Introduction

Delivery by Caesarean Section has become more frequent in recent decades and accounts for over 30% of births in the United States and Europe [1]. There is now growing evidence that Caesarean Section has an impact on the immune system in the offspring [2]. Several studies show immunological alterations at birth or within a few days after Caesarean Section delivery in healthy neonates [3–6]. Such changes may have consequences for the future health of the offspring and alter the risk of disease in adulthood [7–9]. The reports are however, limited to the first week after birth and there is little knowledge of whether Caesarean Section will lead to longer-term effects on the immune responsiveness during infancy and childhood.

Caesarean Section is associated with an increased risk to develop type 1 diabetes [7,10]. Type 1 diabetes is preceded by an asymptomatic period of islet autoimmunity, which often initiates between 9 months and 2 years of life [11,12]. Here, we investigated healthy children at increased familial risk for type 1 diabetes for their immune status during infancy in relation to their mode of delivery. We examined cytokine markers and immune response to vaccination shortly prior to the susceptible age of islet autoimmunity initiation at age 6 months as well as during infancy.

2. Material and methods

2.1. Study subjects and samples

Samples for serum and peripheral blood mononuclear cells (PBMC) were obtained from children participating in the prospective BABYDIET study. The BABYDIET study recruited 150 children carrying high HLA-risk genotypes between 2000 and 2006 to investigate whether delay of exposure to gluten could reduce the risk to develop islet autoimmunity (ClinicalTrials.gov NCT01115621). The intervention failed to show an effect on islet autoantibody development and all participants continued with follow-up examinations within a natural history protocol [13]. Children were followed with 3-monthly venous blood samples starting at the age of 3 months to age 3 years, and yearly thereafter. Information on mode of delivery was collected from each child's pediatric record. Information on tetanus vaccination was regularly provided by the parents as a copy of their child's vaccination certificate, typically at scheduled visits corresponding to blood draw. A detailed description of the study design has been reported [13]. The study was approved by the ethical committee of Bavaria, Germany, (Ludwig-Maximilians University No. 329/00) and executed in accordance with

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the principles of the Declaration of Helsinki, including written informed consent by the parents or guardians of all participating children.

2.2. Multiplex cytokine assay

The concentration of IL-6, IL-8, IL-10, TNF-α, IL-12p70, IL-1β, GM-CSF, IL-2, and IFN-γ was measured in serum collected at the age of six months in 94 of the 150 BABYDIET participants in whom there was sufficient serum remaining at −80 °C. These included 40 children delivered by Caesarean Section and 54 by vaginal delivery. The Human Proinflammatory-9 Ultra-Sensitive Kit from MesoScaleDiscovery (MSD, Gaithersburg, MD) was used for cytokine detection according to the manufacturer's instructions. All standards and samples were measured in duplicate. MSD plates were analyzed on the MESO QUICKPLEX SQ 120 instrument and the data analyzed with the DISCOVERY WORKBENCH® Data Analysis software (MSD).

2.3. $CD4^+$ T cell responses to tetanus toxoid after vaccination

PBMC were isolated within 24 h from venous blood by density gradient centrifugation over Lymphoprep (Axis-Shield) according to the manufacturer's instructions. $CD4^+$ T cells responses to tetanus toxoid were studied at multiple time points during infancy in 26 children, including 15 delivered by Caesarean Section. PBMC were labeled with CFSE (Invitrogen, Carlsbad, CA) as previously described [14]. 2×10^5 cells were added to each well of a round-bottom 96-well microtiter plate and incubated without or with tetanus toxoid (1 μl/ml; Novartis) in RPMI1640 supplemented with 5% heat-inactivated human serum AB (PAA), 2 mM L-glutamine (Lonza) and 100 U/ml Penicillin/Streptomycin (Lonza) at 37 °C, 5% CO_2 and 95% humidity. After five days, the cell cultures were stained for CD4 (PB), CD25 (PE), CD45RA (APC), CD8 (APC-Cy7), CD45RO (PE-Cy7) and 7AAD (live/dead) and processed for flow cytometric analysis.

2.4. Tetanus toxoid antibodies

Antibody responses to tetanus were examined in serum obtained at the age of 1 and 2 years after an expected three and four tetanus vaccinations, respectively. The measurement of tetanus toxoid-specific IgG1, IgG2, and IgG4 was performed by ELISA as previously described [15].

2.5. Immune cell phenotyping

For analysis of memory T cells and regulatory T cells, $3–6 \times 10^5$ PBMC were freshly stained with the following mouse anti-human monoclonal antibodies using standard techniques: CD4 (PerCP), CD25 (PE), CD45RO (APC), CD127 (eFluor450), and FOXP3 (Alexa Fluor 488). Data were acquired on a Becton Dickinson ARIA II flow cytometer with FACS DIVA™ (Version 7.0; Becton Dickinson) acquisition software within 24 h and analyzed with FlowJo Version 7.6.5 (TreeStar Inc.).

2.6. Statistical analyses

Immune phenotype, cytokine measurements and T cell responses were compared between children born by Caesarean Section or vaginal delivery. Using the non-parametric Mann–Whitney U test, we compared both groups for each variable separately, and for the first principal component derived from a principal component analysis based on all measured cytokines. Data were analyzed using GraphPad Prism (GraphPad Prism 4.02; San Diego, CA), SPSS for Windows (SPSS 18.0; Chicago, IL), SAS (SAS 9.3; Cary, NC), and R (R 3.0.3; http://cran.r-project.org/). Uncorrected two-tailed p values <0.05 were considered significant.

3. Results

At age six months, children born by Caesarean Section had lower serum concentrations of IFN-γ (Caesarean Section, median, 1.29 pg/ml; interquartile range [IQR], 0.86–2.01; vaginal birth, median, 1.89 pg/ml; IQR, 1.20–3.06; $p = 0.014$), and IL-8 (Caesarean Section, median, 19.3 pg/ml; IQR, 9.9–62.3; vaginal birth, median, 40.2 pg/ml; IQR, 17.1–587; $p = 0.005$) (Fig. 1A–B). Furthermore, children born by Caesarean Section differed in their overall cytokine response and exhibited a general hypo-inflammatory response compared to children born by vaginal delivery as indicated by the principal component analysis ($p = 0.019$; Fig. 1C; Suppl. Table 1).

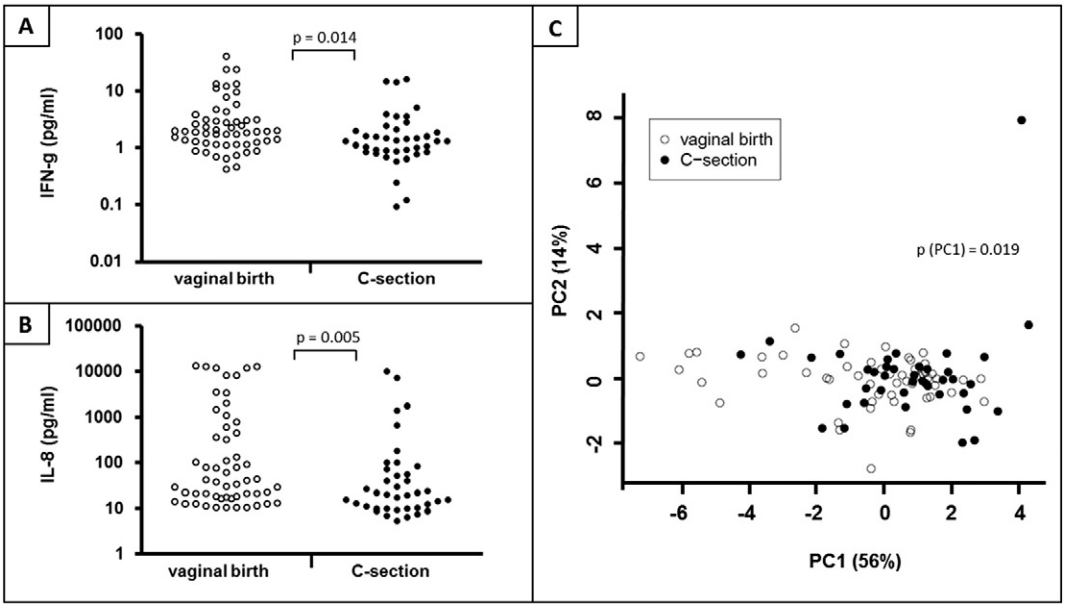

Fig. 1. Serum cytokine concentration in six-months old children. (A) Concentration of IFN-γ and (B) IL-8 and (C) biplot of the first two principal components derived from a principal component analysis of all measured cytokines (IL-6, IL-8, IL-10, TNF-α, IL-12p70, IL-1β, GM-CSF, IL-2, and IFN-γ) with the proportion of explained variance by each principal component in brackets. Children are stratified into those born by vaginal delivery (open circles, n = 54) and those born by Caesarean Section (filled circles, n = 40). p values were calculated using Mann–Whitney U tests.