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Abstract

Immune dysregulation during pregnancy may influence behavior and neurodevelopment in offspring, but few human studies have tested this hypothesis. Using structural equation modeling, we examined associations between maternal inflammatory markers at 28 weeks gestation and child neurodevelopmental outcomes at 20 months of age in a sample of 1,453 mother-child pairs. We observed several associations between maternal inflammatory markers measured in the late second or early third trimester and child neurodevelopmental outcomes. The direction of association for some markers was unexpected. Further research is warranted to confirm and elucidate the exact nature of these findings.

Keywords: inflammatory markers; developmental outcomes; children; pregnancy

1. Introduction

During pregnancy, a fundamental feature of the immune system is to regulate the balance between protection of both the mother and developing fetus against pathogens present in their environment, and maintaining tolerance of the fetal allograft (Aghaeepour et al., 2017). In normal pregnancy, each trimester is marked by changes in immunological functioning. In the first trimester Th1-type immunity is predominant, characterized by cell-mediated inflammatory reactions and production of pro-inflammatory responses (e.g., IL-1β, IL-2, IFN-γ, TNF-α) (Sargent et al., 2006; Wegmann et al., 1993). In the second and early-third trimesters, a shift is observed toward Th2-type immunity, characterized by B-cell activation and production of antibodies and anti-inflammatory responses (e.g., IL-4, IL-5, IL-10) (Sargent et al., 2006; Wegmann et al., 1993). During the mid- to late-third trimester, the renewed pro-inflammatory (Th1) state takes over to promote contraction of the uterus and prepare for delivery of the baby and placenta (Racicot et al., 2014). The Th1/Th2 ratio is commonly used to characterize dominating cytokine patterns, although this ratio may not fully capture variations in immune responses (Kidd, 2003). In addition to the Th1 and Th2 cytokines, chemokines, angiogenesis markers, and C-reactive protein (CRP) each play a role in the multifaceted and complex processes that comprise the immune system during pregnancy (Szarka et al., 2010).

Experimental animal research indicates that the maternal gestational immune response is important for successful pregnancy and positive birth and developmental outcomes, and this is hypothesized to also be true in humans (Chau et al., 2016). Experimental murine studies show that prenatal inflammation can cause both immediate and lasting changes in behavior and CNS structure and function in offspring, impairing working memory, spatial learning, locomotor activity, and startle reactivity (see Boksa, 2010, for a review). When looking at specific

inflammatory cells in pregnant rats at the beginning of the human equivalent of the third trimester, Gilmore and colleagues (2004) found that higher levels of the cytokine IL-6, which is considered to have both pro- and anti-inflammatory properties, and the pro-inflammatory cytokines IL-1β and TNF-α reduce the number of primary dendrites that develop and diminish the complexity of embryonic cortical neurons.

Human research on the effects of maternal immune response on later child developmental outcomes is limited but suggests that maternal immune activation can hinder neurodevelopment. Higher levels of maternal mid-gestational IL-1, TNF-α, and IL-6 increase the risk of intraventricular hemorrhage and neonatal white matter damage (Dammann and Leviton, 1997), although the mechanisms that underlie this association are not well understood (Smith et al., 2007). Studies have mostly examined maternal infections or autoimmune conditions rather than specific cytokines or other inflammatory markers, and studies have focused on autism spectrum disorders (ASD) and mental disorders rather than more subtle measures of neurodevelopment (Brown, 2012; Jiang et al., 2016). Nevertheless, most relevant to the current study, several case-control studies have reported associations between specific prenatal cytokine and chemokine concentrations and ASD risk. Studies in which inflammatory markers were measured at early or mid-gestation reported an increased risk of ASD with increasing serum concentrations of IFN-γ, IL-2, IL-4, and IL-6 (Goines et al., 2011), MCP-1, IL-4, IL-10, TNF- α, and TNF-β (Abdallah et al., 2013, 2012), IFN-γ, IL-1α, and IL-6 (Jones et al., 2017), and C-reactive protein (CRP) (Brown et al., 2014). In contrast, a recent study reported an increased risk of ASD with lower maternal CRP markers (Zerbo et al., 2016), and mothers of children with ASD (without intellectual disability) or developmental delay had lower IL-8 and MCP-1 concentrations than controls (Jones et al., 2017). Thus, while psychoneuroimmunology studies suggest that the

prenatal immune system is linked to brain and nervous system development, potentially resulting in later behavioral and psychopathological outcomes, further research is needed to elucidate the exact nature of these complex relationships (O'Connor et al., 2014).

The Seychelles Child Development Study Nutrition Cohort 2 (SCDS NC2) is a large, well-characterized prospective birth cohort (N = 1535) from the Republic of Seychelles that was designed to evaluate whether prenatal exposure to methylmercury and nutrients from high fish consumption during pregnancy influences child development (Strain et al., 2015), and whether pro- and anti-inflammatory markers play a role in these associations. In this analysis, we examined complex maternal gestational cytokine profiles as well as chemokines, CRP, and markers of angiogenesis in relation to various developmental outcomes to further our understanding of the role of maternal immune dysregulation in neurodevelopment. We hypothesized that pro-inflammatory (Th1) markers would be adversely associated with developmental outcomes and anti-inflammatory (Th2) markers would be beneficially associated.

2. Methods

2.1 Study Population

SCDS NC2 has been described in detail elsewhere (Strain et al., 2015). In short, 1,535 pregnant women were recruited from 2008 to 2011 during their first antenatal visit (from 14 weeks gestation). Inclusion criteria for NC2 included being native Seychellois, being ≥16 years of age, having a singleton pregnancy, and having no obvious health concerns (Strain et al., 2015). Children were tested for developmental outcomes at about 20 months of age. They were included in the current sample if they had available developmental assessment data and data from an assay of inflammatory markers in maternal serum at 28 weeks gestation, and relevant covariates (see Exclusions). The study was reviewed and approved by the Seychelles Ethics Board and the Research Subjects Review Board at the University of Rochester.

2.2 Exclusions

The following exclusion criteria were applied for the current study: birthweight <1500 g (n = 6), pre- or perinatal death (n = 20), maternal pre- or perinatal complications (n = 3), head trauma with loss of consciousness (n = 3), seizures (n = 10), disability (n = 1), and twin births (n = 34). An additional participant was also removed after withdrawing consent to participate in the study. Also, inflammatory markers vary throughout pregnancy (Sargent et al., 2006; Wegmann et al., 1993), thus two participants were removed because their serum samples were obtained at gestational ages (18 and 40 weeks) that varied substantially from the rest of the cohort (20 – 33 weeks, interquartile range (IQR) 27 – 28 weeks). Finally, two mothers with unusually high values for most inflammatory markers measured here were excluded from all analyses. As such, our study population consisted of mothers and children without apparent clinical problems. The

total sample size after exclusions was 1408, corresponding to the number of participants with data for at least one inflammatory marker.

2.3 Developmental assessment

At around 20 months of age (mean 20 months (*SD* = 1.4), IQR = 20 – 21 months, range: 15-32 months), children were examined by trained nurses using the following measures: (1) Bayley Scales of Infant Development II (BSID-II), a commonly used developmental test that yields two standard scores reflecting children's current cognitive (MDI) and psychomotor skills (PDI); (2) MacArthur-Bates Communicative Development Inventory (CDI), a standardized parental survey that yields separate raw scores for Words Produced, Words Understood, and Total Gestures; and (3) the Infant Behavior Questionnaire – Revised (IBQ-R), which is a parent questionnaire that assesses child temperament. We used raw scores from the three IBQ-R subscales: surgency (a dimension of temperament in which a person tends towards extraversion, sociability and impulsivity), negative affect, and effortful control.

2.4 Inflammatory Markers

Non-fasting blood samples were collected at approximately 28 weeks of gestation (mean 28 weeks (*SD* = 1.1)). Samples were collected by antecubital venipuncture into evacuated serum tubes. They were placed on water ice and allowed to sit for 30 minutes before being centrifuged at 2500 rpm for 15 minutes. Aliquots were shipped to Ulster at -80 °C and stored until analysis. Analysis of serum inflammatory markers took place approximately five years after sample collection, and were assayed via Meso Scale Discovery (MSD) multiplex assay. Freeze thaw cycles were avoided by using a fresh unthawed aliquot for each participant ID. This would minimize any effect of long-term storage on cytokine concentrations, as all samples were treated the same.

We selected a panel of inflammatory markers for assessment in maternal serum samples based on reported associations with methylmercury exposure and n-3 long-chain polyunsaturated fatty acid status (the original predictors of interest for developmental outcomes in the NC2 study) (Nyland et al., 2011b, 2011a), or because they are believed to play a role in fetal brain development (Smith et al., 2007). We quantified cytokines from the two major subsets of T helper (Th) cells, Th1 and Th2. Pro-inflammatory cytokines associated with cell-mediated reactions (Th1) included interleukin (IL)-1β, IL-2, interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α). Anti-inflammatory cytokines associated with B-cell activation and antibody production (Th2) included IL-4, IL-5, and IL-10. IL-6, which has both pro- and anti-inflammatory roles (Diehl and Rincón, 2002), was also measured. Additional inflammatory markers included C-reactive protein (CRP); the chemokines monocyte chemoattractant protein 1 (MCP-1) and thymus and activation regulated chemokine (TARC); and the angiogenesis markers soluble fms-like tyrosine kinase 1 (sFlt-1) and vascular endothelial growth factor D (VEGF-D). The individual biomarkers were analyzed as continuous variables measured in pg/mL, except CRP which was measured in mg/L.

Values below the lower limit of detection (LLOD) for each marker were imputed as LLOD/2 (see Table 2 for LLOD values, percent detected for each analyte, and inter- and intra-assay coefficients of variation). Two markers had a substantial amount (> 20%) of undetectable values: IL-2 (LLOD: 0.09 pg/mL; 36.1% detected) and IL-4 (LLOD: 0.02 pg/mL; 35.5% detected). Excluding IL-2 and IL-4 did little to impact the relationships with developmental outcomes, therefore, we kept these markers in the analyses.

2.5 Covariates

As in Strain et al. (2015), our models adjusted for the following variables thought to impact child development as covariates in the regression models: maternal age, child age at testing (in months), child sex, Hollingshead socioeconomic status (SES; possible range 8 – 66, with higher scores indicating higher SES), and number of parents living with the child (0 – 2). Maternal age, child age at testing (in months), and Hollingshead socioeconomic status were treated as continuous variables.

2.6 Statistical Analyses

First, we calculated measures of central tendency and variance to describe demographics, developmental characteristics, and inflammatory markers in mothers and children (see Tables 1 and 2). Subsequently, we examined the presence of influential points (Cook's distances larger than 0.50) or statistical outliers (standardized residuals in excess of ± 3) in each model as defined previously (van Wijngaarden et al., 2013). No influential points were identified, but a small number of statistical outliers were identified (ranging from 0 to 13 depending on the model). Models were run with and without these values. The interpretation of results with and without outliers remained the same (data not shown), and thus we present the results here using the full dataset including outliers.

Then, to identify whether there was any natural clustering among the inflammatory marker variables, an exploratory factor analysis with geomin oblique rotation was conducted with Mplus, version 8 (Muthén and Muthén, 1998-2017). Full-information maximum likelihood (FIML) with robust standard errors was used as the estimator to address missing inflammatory marker data ($N = 1416$). This is considered the ideal approach when conducting analyses with incomplete and nonnormal data (Newman, 2014; Yuan, 2009). The fit of each model was examined via the magnitude and p values of loadings, and model fit statistics such as the Root

Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), and Bayesian Information Criterion (BIC). Variables with non-significant rotated factor loadings were removed and models re-run until all remaining factor loadings were significant and model fit statistics were satisfactory. It was hypothesized that two factors would emerge, representing pro- or anti-inflammatory activity.

The identified factors were then modeled as latent factors in regression models predicting each developmental outcome at 20 months, after adjustment for maternal age, child age at testing, child sex, Hollingshead socioeconomic status, and number of parents living with the child. Then, additional regression models were run with the 13 individual biomarkers (IL-1β, IL-2, IFN-γ, TNF-α, IL-4, IL-5, IL-6, IL-10, MCP-1, TARC, sFlt-1, VEGF-D, CRP) predicting each developmental outcome at 20 months (BSID-II PDI and MDI; MacArthur-Bates CDI Words Produced, Words Understood, and Total Gestures; and IBQ-R Surgency, Negative Affect, and Effortful Control), adjusting for the same covariates as before. FIML with robust standard errors was again used as the estimator ($N = 1453$). We confirmed that collinearity (e.g. variance inflation factors >10, correlations >0.9) between the inflammatory markers was not an issue in these regression models. Moreover, in secondary analyses we used categorical inflammatory marker variables (with categories based on bands of multiples of 1 SD above and below the mean for each variable, using the lowest category as the reference group) to further examine dose-response patterns and determine the consistency of results. Interpretation of the findings was not materially different to that using the continuous inflammatory marker variables, and categorical results were therefore not further presented. Analyses were conducted with Mplus, version 8 (Muthén and Muthén, 1998-2017).

3. Results

Summary statistics for the participants and variables included in the study are presented in Tables 1 and 2. Mothers were on average about 27 years old. The sample included more boys than girls (52.1%). Most children (1008, 73.3%) reportedly lived with both parents, whereas 352 (25.6%) lived in a one-parent household, and 15 (1.1%) did not live with either parent. The average Hollingshead SES was near the midpoint of the scale, with scores ranging from very low to very high SES.

Exploratory factor analysis with geomin oblique rotation was conducted to determine whether there were distinct clusters, or factors, that arise within the group of inflammatory markers, reflective of the complex functions and interrelations within the immune system. It was hypothesized that two factors would emerge, representing pro- or anti-inflammatory activity. Full-information maximum likelihood (FIML) with robust standard errors was used as the estimator for analyses conducted in Mplus. In line with our hypothesis, the model with two extracted factors was the best fit to the data. Contrary to hypotheses, however, these factors were not strictly pro- and anti-inflammatory in nature (see Table 3 for a summary of the factor analysis results). The mostly pro-inflammatory factor was comprised of IL-1β, CRP, IL-6, MCP-1, and sFlt-1, and the mostly anti-inflammatory factor was comprised of IL-4, IL-5, IL-10, IL-2, IFN-γ, and TNF-α. In the initial run of the two-factor model, TARC and VEGF-D were not loading on either factor and thus were removed for the final model run. The final two-factor model had an RMSEA of .04 (90% CI: .03 - .05) and CFI of .78, indicating acceptable model fit. The *p* value representing whether there is a statistically significant improvement in fit for the model with one more factor indicated that the two-factor model was a better fit than the one-factor model ($p < .001$). This test also indicated that the three-factor model was a better fit than

the two-factor model ($p < .001$), but the three-factor model had a slightly higher BIC value (44071.51 versus 44061.70 for the two-factor model; lower BIC is preferred), and the third factor was only comprised of two inflammatory markers (IFN-γ and CRP) that were cross-loading on the anti- and pro-inflammatory factors, respectively. Thus, the two-factor model was retained for further analyses.

The two factors identified from the exploratory factor analysis were then modeled as latent factors in structural equation models predicting each developmental outcome at 20 months, after adjustment for maternal age, child age at testing, child sex, Hollingshead socioeconomic status, and number of parents living with the child (see Table 4). The latent pro- and anti-inflammatory factors were largely unrelated to developmental outcomes at 20 months, with the exception of a modest association between the anti-inflammatory factor and psychomotor development (PDI) scores (B [95% CI] = -2.66 [-5.07, -0.25]; $p = .03$), such that an overall increase in maternal anti-inflammatory activity at 28 weeks gestation was modestly associated with worse psychomotor development outcomes at 20 months.

Then, additional regression models were run with all continuous inflammatory marker variables together in a path model predicting each developmental outcome at 20 months, adjusting for the same covariates as before (see Figures 1, 2, and 3). Across all models, increased TARC was significantly associated with better psychomotor development (PDI; B [95% CI] = 0.44 [0.22, 0.66]; $p < .001$), more use of gestural communication (B [95% CI] = 0.61 [0.33, 0.89]; $p < .001$), increased negative affect (B [95% CI] = 0.03 [0.01, 0.06]; $p = .005$), and better effortful control (B [95% CI] = 0.05 [0.02, 0.07]; $p < .001$); increased VEGF-D was significantly associated with better psychomotor development (B [95% CI] = 0.37 [0.08, 0.66]; $p = .01$), better receptive language skills (B [95% CI] = 3.82 [1.10, 6.54]; $p = .006$), and more use of gestural

communication (*B* [95% CI] = 0.37 [0.14, 0.60]; *p* = .002); increased IL-1β was associated with decreased negative affect (*B* [95% CI] = -0.10 [-0.17, -0.03]; *p* = .004) and better effortful control (*B* [95% CI] = 0.06 [0.004, 0.11]; *p* = .036); increased IL-2 was significantly associated with worse psychomotor development (*B* [95% CI] = -0.96 [-1.85, -0.07]; *p* = .03); increased IL-10 was associated with less use of gestural communication (*B* [95% CI] = -0.18 [-0.31, -0.04]; *p* = .01); and increased sFlt-1 was associated with increased negative affect (*B* [95% CI] = 0.06 [0.01, 0.10]; *p* = .009). No other inflammatory marker significantly predicted neurodevelopmental outcomes at 20 months.

As previously reported (Strain et al., 2015), associations between child, maternal, and familial covariates and developmental outcomes, when present, were in the expected directions.

4. Comments

We studied the association of maternal markers of inflammation with children's neurodevelopmental scores and hypothesized pro-inflammatory markers to be associated with poorer child performance and anti-inflammatory markers to be associated with better performance on the developmental assessments at 20 months. We found significant beneficial associations for pro-inflammatory markers TARC and IL-1β, and pro-lymphangiogenic VEGF-D, and adverse associations for pro-inflammatory IL-2, anti-inflammatory IL-10, and anti-angiogenic sFlt-1. In addition, the mostly anti-inflammatory factor identified by the exploratory factor analysis (comprised of IL-2, IL-4, IL-5, IL-10, IFN-γ, and TNF-α) was adversely associated with psychomotor development. Thus, few of the significant associations we found were in the expected directions.

IL-2 and sFlt-1 each demonstrated an adverse association, with psychomotor development and increased negative affect, respectively. In a small case-control study, increased

IL-2 concentrations were associated with an increased risk of autism spectrum disorder (Goines et al., 2011), which itself is associated with motor deficits (Bo et al., 2016). In addition, another study found that higher levels of sFlt-1 during mid-pregnancy are associated with poorer neonatal outcomes (Masoura et al., 2014), The rest of the significant inflammatory markers, however, demonstrated unexpected associations with child developmental outcomes. IL-1β had unexpected beneficial associations with reduced negative affect and better effortful control, but in concentrations reflecting physiological and not inflammatory amounts, IL-1β has been found to have neuroprotective effects and is important for normal brain function (Hewett et al., 2015).

Despite the statistically significant findings in the current study, these associations were modest in magnitude and no clear patterns of associations emerged. Thus, the results of this study do not support a strong link between the maternal gestational immune markers assayed and child neurodevelopmental outcomes. While the unexpected directions of some of our associations currently have no clear biological explanation and may simply be false positives, the maternal immune response during pregnancy is complex and little is known about its relationship with developmental outcomes in children. Therefore, our associations necessitate confirmation and further clarification.

This study has important strengths, in particular its use of a well-controlled, large prospective birth cohort of children, the relatively large panel of inflammatory markers, and the use of humans as opposed to animals, all of which are rare in the study of maternal immune activation and child outcomes. Nevertheless, our study also has several limitations, which may have limited our ability to observe clear patterns of association. Levels of inflammatory markers fluctuate throughout pregnancy (Sargent et al., 2006; Wegmann et al., 1993). Concentrations at 28 weeks gestation could vary from concentrations at other time points that were not measured in

the current study but could be more strongly associated with developmental endpoints. Although we selected a broad panel of inflammatory markers, there are others that we did not study. Furthermore, the current study lacked information that could have aided in the interpretation of our results or affected levels of inflammatory markers and child developmental outcomes, including data on whether the mothers had specific inflammatory or autoimmune conditions, whether they were exposed to certain infectious agents during pregnancy, maternal pregnancy BMI, breastfeeding, and the health of the child. However, we excluded mothers and children with certain clinical conditions. Moreover, measurable levels of inflammatory markers can be affected by the time samples spent in storage (in this study, approximately five years), possibly due to cytokine degradation (de Jager et al., 2009; Parkitny et al., 2013; Zhou et al., 2010). However, all samples in the current study underwent the same conditions of freezing, storage, and thawing, and we would not expect the impact of storage on inflammatory marker concentrations to vary based on neurodevelopment outcomes or other variables in the model. Additionally, blood draws generally took place in the morning, but record of the specific time at which each blood draw occurred is only available for 406 participants. The current study was therefore unable to account for any potential diurnal rhythms in the inflammatory markers; however, of the 406 blood draws, 364 (90%) took place before noon, and 42 (10%) took place between noon and 3pm, so we would not expect diurnal rhythms to vary significantly nor be associated with any of the developmental outcomes. Finally, inherent in any study of developmental outcomes in infants, the dependent variables are less sensitive and specific than measures of child development at older ages (Mahaffey, 1998); and the precision of the independent biomarkers is in sharp contrast to these endpoint measures. Further research at older

ages will be necessary to capture more robust developmental endpoints and to clarify the ideal balance of pro- versus anti-inflammatory cytokines and chemokines throughout pregnancy.

In conclusion, while several associations were observed between inflammatory markers measured around 28 weeks of gestation and neurodevelopmental outcomes at 20 months of age, the direction of these associations was not consistent. Our findings could reflect the true absence of associations in a relatively healthy population of mothers and children, or be the result of methodological limitations preventing us from detecting subtle associations. Further study is required using measurements of maternal inflammatory markers at various points throughout gestation to examine whether differences in absolute amounts or trajectories of inflammatory markers are associated with child neurodevelopmental outcomes.

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References

Abdallah, M.W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E.L., Hougaard, D.M., 2013. Amniotic fluid inflammatory cytokines: Potential markers of immunologic dysfunction in autism spectrum disorders. World J. Biol. Psychiatry 14, 528–538. doi:10.3109/15622975.2011.639803

Abdallah, M.W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E.L., Hougaard, D.M., 2012. Amniotic fluid chemokines and autism spectrum disorders: An exploratory study utilizing a Danish Historic Birth Cohort. Brain. Behav. Immun. 26, 170–176. doi:10.1016/j.bbi.2011.09.003

Aghaeepour, N., Ganio, E.A., Mcilwain, D., Tsai, A.S., Tingle, M., Van Gassen, S., Gaudilliere, D.K., Baca, Q., McNeil, L., Okada, R., Ghaemi, M.S., Furman, D., Wong, R.J., Winn, V.D., Druzin, M.L., El-Sayed, Y.Y., Quaintance, C., Gibbs, R., Darmstadt, G.L., Shaw, G.M., Stevenson, D.K., Tibshirani, R., Nolan, G.P., Lewis, D.B., Angst, M.S., Gaudilliere, B., 2017. An immune clock of human pregnancy. Sci. Immunol. 2, eaan2946. doi:10.1126/sciimmunol.aan2946

Bo, J., Lee, C.M., Colbert, A., Shen, B., 2016. Do children with autism spectrum disorders have motor learning difficulties? Res. Autism Spectr. Disord. 23, 50–62. doi:10.1016/j.rasd.2015.12.001

Boksa, P., 2010. Effects of prenatal infection on brain development and behavior: A review of findings from animal models. Brain. Behav. Immun. 24, 881–897. doi:10.1016/j.bbi.2010.03.005

Brown, A.S., 2012. Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. Dev. Neurobiol. 72, 1272–1276. doi:10.1002/dneu.22024

Brown, A.S., Sourander, A., Hinkka-Yli-Salomäki, S., McKeague, I.W., Sundvall, J., Surcel, H.-M., 2014. Elevated maternal C-reactive protein and autism in a national birth cohort. Mol. Psychiatry 19, 259–264. doi:10.1038/mp.2012.197

Chau, A., Markley, J.C., Juang, J., Tsen, L.C., 2016. Cytokines in the perinatal period - Part II. Int. J. Obstet. Anesth. 26, 48–58. doi:10.1016/j.ijoa.2015.12.006

Dammann, O., Leviton, A., 1997. Maternal Intrauterine Infection, Cytokines, and Brain Damage in the Preterm Newborn. Pediatr. Res. 42, 1–8. doi:10.1203/00006450-199707000-00001

de Jager, W., Bourcier, K., Rijkers, G.T., Prakken, B.J., Seyfert-Margolis, V., 2009. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC Immunol. 10, 52. doi:10.1186/1471-2172-10-52

Diehl, S., Rincón, M., 2002. The two faces of IL-6 on Th1/Th2 differentiation. Mol. Immunol. 39, 531–536. doi:10.1016/S0161-5890(02)00210-9

Gilmore, J.H., Fredrik Jarskog, L., Vadlamudi, S., Lauder, J.M., 2004. Prenatal infection and risk for schizophrenia: IL-1beta, IL-6, and TNFalpha inhibit cortical neuron dendrite development. Neuropsychopharmacology 29, 1221–9. doi:10.1038/sj.npp.1300446

Giuliani, F., Vernay, A., Leuba, G., Schenk, F., 2009. Decreased behavioral impairments in an Alzheimer mice model by interfering with TNF-alpha metabolism. Brain Res. Bull. 80, 302–308. doi:10.1016/j.brainresbull.2009.07.009

Goines, P.E., Croen, L.A., Braunschweig, D., Yoshida, C.K., Grether, J., Hansen, R., Kharrazi, M., Ashwood, P., Van de Water, J., 2011. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Mol. Autism 2, 13. doi:10.1186/2040-2392-2-13

Hewett, S.J., Jackman, N.A., Claycomb, R.J., Neuroscience, P.I., Haven, N., 2015. Interleukin-

1β in Central Nervous System Injury and Repair Sandra. Eur J Neurodegener Dis 1, 195–211. doi:10.1016/j.ygyno.2014.12.035.Pharmacologic

Jiang, H.-Y., Xu, L.-L., Shao, L., Xia, R.-M., Yu, Z.-H., Ling, Z.-X., Yang, F., Deng, M., Ruan, B., 2016. Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. Brain. Behav. Immun. 58, 165–172. doi:10.1016/j.bbi.2016.06.005

Jones, K.L., Croen, L.A., Yoshida, C.K., Heuer, L., Hansen, R., Zerbo, O., DeLorenze, G.N., Kharrazi, M., Yolken, R., Ashwood, P., Van de Water, J., 2017. Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. Mol. Psychiatry 22, 273–279. doi:10.1038/mp.2016.77

Kidd, P., 2003. Th1/Th2 balance: The hypothesis, its limitations, and implications for health and disease. Altern. Med. Rev. 8, 223–246.

Mahaffey, K.R., 1998. Methylmercury exposure and neurotoxicity. JAMA 280, 737–738. doi:10.1001/jama.280.8.737

Masoura, S., Kalogiannidis, I., Makedou, K., Theodoridis, T., Koiou, K., Gerou, S., Athanasiadis, A., Agorastos, T., 2014. Biomarkers of endothelial dysfunction in preeclampsia and neonatal morbidity: A case-control study. Eur. J. Obstet. Gynecol. Reprod. Biol. 175, 119–123. doi:10.1016/j.ejogrb.2014.01.012

Muthén, L.K., Muthén, B.O., 1998. Mplus User's Guide. Eighth Edition. Muthén & Muthén, Los Angeles, CA. doi:10.1111/j.1600-0447.2011.01711.x

Newman, D.A., 2014. Missing data: Five practical guidelines. Organ. Res. Methods 17, 372–411. doi:10.1177/1094428114548590

Nyland, J.F., Fillion, M., Barbosa, F., Shirley, D.L., Chine, C., Lemire, M., Mergler, D.,

Silbergeld, E.K., 2011a. Biomarkers of methylmercury exposure immunotoxicity among fish consumers in amazonian Brazil. Environ. Health Perspect. 119, 1733–1738. doi:10.1289/ehp.1103741

Nyland, J.F., Wang, S.B., Shirley, D.L., Santos, E.O., Ventura, A.M., de Souza, J.M., Silbergeld, E.K., 2011b. Fetal and maternal immune responses to methylmercury exposure: A cross-sectional study. Environ. Res. 111, 584–589. doi:10.1016/j.envres.2011.02.010

O'Connor, T.G., Moynihan, J.A., Caserta, M.T., 2014. Annual research review: The neuroinflammation hypothesis for stress and psychopathology in children - Developmental psychoneuroimmunology. J. Child Psychol. Psychiatry Allied Discip. 55, 615–631. doi:10.1111/jcpp.12187

Parkitny, L., McAuley, J.H., Kelly, P.J., Di Pietro, F., Cameron, B., Moseley, G.L., 2013. Multiplex cytokine concentration measurement: How much do the medium and handling matter? Mediators Inflamm. doi:10.1155/2013/890706

Racicot, K., Kwon, J.Y., Aldo, P., Silasi, M., Mor, G., 2014. Understanding the complexity of the immune system during pregnancy. Am. J. Reprod. Immunol. 72, 107–116. doi:10.1111/aji.12289

Sargent, I.L., Borzychowski, A.M., Redman, C.W.G., 2006. NK cells and human pregnancy - an inflammatory view. Trends Immunol. 27, 399–404. doi:10.1016/j.it.2006.06.009

Shen, Y., Liu, S.S., Zhan, M.Y., Luo, J.H., Zhu, L.J., 2010. Interleukin-2 enhances dendritic development and spinogenesis in cultured hippocampal neurons. Anat. Rec. 293, 1017–1023. doi:10.1002/ar.21118

Smith, S.E.P., Li, J., Garbett, K., Mirnics, K., Patterson, P.H., 2007. Maternal immune activation alters fetal brain development through interleukin-6. J. Neurosci. 27, 10695–10702.

doi:10.1523/JNEUROSCI.2178-07.2007

Strain, J.J., Yeates, A.J., van Wijngaarden, E., Thurston, S.W., Mulhern, M.S., McSorley, E.M., Watson, G.E., Love, T.M., Smith, T.H., Yost, K., Harrington, D., Shamlaye, C.F., Henderson, J., Myers, G.J., Davidson, P.W., 2015. Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: Associations with child development at 20 mo of age in an observational study in the Republic of Seychelles. Am. J. Clin. Nutr. 101, 530–537. doi:10.3945/ajcn.114.100503

Szarka, A., Rigó, J., Lázár, L., Beko, G., Molvarec, A., 2010. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol. 11, 1–9. doi:10.1186/1471-2172-11-59

van Wijngaarden, E., Thurston, S.W., Myers, G.J., Strain, J.J., Weiss, B., Zarcone, T., Watson, G.E., Zareba, G., McSorley, E.M., Mulhern, M.S., Yeates, A.J., Henderson, J., Gedeon, J., Shamlaye, C.F., Davidson, P.W., 2013. Prenatal methyl mercury exposure in relation to neurodevelopment and behavior at 19 years of age in the Seychelles Child Development Study. Neurotoxicol. Teratol. 39, 19–25. doi:10.1016/j.ntt.2013.06.003

Wegmann, T.G., Lin, H., Guilbert, L., Mosmann, T.R., 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a TH2 phenomenon? Immunol. Today 14, 353–356. doi:10.1016/0167-5699(93)90235-D

Yuan, K.-H., 2009. Normal distribution based pseudo ML for missing data: With applications to mean and covariance structure analysis. J. Multivar. Anal. 100, 1900–1918. doi:10.1016/j.jmva.2009.05.001

Zerbo, O., Traglia, M., Yoshida, C., Heuer, L.S., Ashwood, P., Delorenze, G.N., Hansen, R.L., Kharrazi, M., Van de Water, J., Yolken, R.H., Weiss, L.A., Croen, L.A., 2016. Maternal

mid-pregnancy C-reactive protein and risk of autism spectrum disorders: The early markers for autism study. Transl. Psychiatry 6, e783. doi:10.1038/tp.2016.46

Zhou, X., Fragala, M.S., McElhaney, J.E., Kuchel, G.A., 2010. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. Curr. Opin. Clin. Nutr. Metab. Care 13, 541–547. doi:10.1097/MCO.0b013e32833cf3bc

Table 1

Summary statistics for maternal inflammatory markers at 28 weeks gestation, infant developmental outcomes at 20 months of age, and model covariates

	n	*M* (*SD*) or *n* (%)	Minimum	Median	Maximum
Developmental Outcomes					
BSID-II MDI, scaled score	1385	87.59 (10.70)	49	88	118
BSID-II PDI, scaled score	1383	96.71 (10.54)	49	97	136
CDI Vocabulary Produced	1411	122.47 (88.19)	0	109	395
CDI Vocabulary Understood	1411	233.48 (91.39)	0	228	396
CDI Total Gestures	1411	46.55 (8.30)	2	47	63
IBQ-R Surgency	1410	5.39 (0.57)	2.15	5.46	7
IBQ-R Negative Affect	1410	4.12 (1.00)	1	4.17	7
IBQ-R Effortful Control	1410	5.19 (0.55)	1	5.25	7
Covariates					
Child sex (male or female)	1448	757 (52.3%) males			
Mother's age at enrollment	1451	26.86 (6.30)	16.03	25.89	46.56
Child's age at visit (months)	1411	20.27 (1.44)	15	20	32
Number of parents living with the child	1411	1030 (73.8%) 2 parents	1		2
Hollingshead SES	1411	31.97 (10.40)	11	31.50	63

Note. Descriptive analyses presented are using raw, untransformed variables. BSID-II: Bayley Scales of Infant Development II; MDI: Mental Development Index; PDI: Psychomotor Developmental Index; CDI: MacArthur-Bates Communicative Development Inventory; IBQ-R: Infant Behavior Questionnaire-Revised. SES: socioeconomic status.

Table 2

Summary statistics and coefficients of variation for maternal inflammatory markers at 28 weeks gestation

Inflammatory Marker	*n*	*M* (*SD*)	LLOD[c] (% detected)	Inter-Assay CV (%)	Intra-Assay CV (%)
IL-1β	1408	0.32 (0.62)	0.04 (82.6%)	44.38	<26
IL-2	1408	0.27 (0.59)	0.09 (36.1%)	25.46	<35
IFN-γ	1408	5.59 (22.05)	0.20 (84.4%)	22.29	<20
TNF-α	1408	7.18 (7.18)	0.04 (100%)	16.14	<22
IL-4	1408	0.14 (0.55)	0.02 (35.5%)	15.28	<30
IL-5	1408	1.34 (2.19)	0.22 (100%)	11.87	<17
IL-10	1408	1.55 (7.22)	0.03 (90.6%)	16.48	<20
IL-6	1408	1.01 (1.62)	0.06 (88.7%)	22.96	<28
MCP-1	1408	74.89 (88.67)	0.09 (100%)	7.72	<20
TARC	1408	100.51 (138.75)	0.22 (100%)	22.2	<15
sFlt-1	1408	2069.69 (1378.88)	0.56 (100%)	12.54	<22
VEGF-D	1408	738.58 (372.06)	2.53 (100%)	17.41	<15
CRP	1379	3.52 (2.71)	1 (98.9%)	3.5	<1.8

Note. Serum biomarkers measured in pg/mL, except CRP which was measured in mg/L. LLOD: lower limit of detection. CV: coefficient of variation.

Table 3

Results of exploratory factor analysis with continuous inflammatory marker variables at 28 weeks gestation ($N = 1416$)

Biomarker	Geomin Rotated Standardized Factor Loadings	
	Factor 1 (Pro-Inflammatory)	Factor 2 (Anti-Inflammatory)
IL-1β	.40	
IL-6	.87	
MCP-1	.47	
sFlt-1	.22	
CRP	.22	
IL-2		.56
IFN-γ		.26
TNF-α		.41
IL-4		.39
IL-5		.28
IL-10		.13

Note. All loadings are geomin rotated standardized factor loadings significant at the $p < .05$ level.

Table 4

Pro-inflammatory versus anti-inflammatory latent factors[a], comprised of continuous inflammatory markers at 28 weeks gestation, predicting developmental outcomes at 20 months of age (N = 1453)

Developmental Outcome		Latent Factor	*B* [95% CI][b]
Bayley Scales of Infant Development-II	Mental Development Index	Pro-Inflammatory	1.23 [-1.31, 3.78]
		Anti-Inflammatory	-1.94 [-4.32, 0.44]
	Psychomotor Development Index	Pro-Inflammatory	-0.43 [-3.16, 2.30]
		Anti-Inflammatory	**-2.66 [-5.07, -0.25]***
MacArthur-Bates CDI	Vocabulary Produced	Pro-Inflammatory	10.49 [-12.86, 33.83]
		Anti-Inflammatory	-12.15 [-33.98, 9.69]
	Vocabulary Understood	Pro-Inflammatory	-0.05 [-19.67, 19.58]
		Anti-Inflammatory	-19.36 [-47.66, 8.93]
	Total Gestures	Pro-Inflammatory	0.40 [-1.46, 2.26]
		Anti-Inflammatory	-0.83 [-3.72, 2.06]
Infant Behavior Questionnaire - Revised	Surgency	Pro-Inflammatory	0.02 [-0.16, 0.20]
		Anti-Inflammatory	-0.08 [-0.29, 0.13]
	Negative Affect	Pro-Inflammatory	0.06 [-0.19, 0.31]
		Anti-Inflammatory	-0.16 [-0.46, 0.15]
	Effortful Control	Pro-Inflammatory	0.03 [-0.15, 0.20]
		Anti-Inflammatory	0.06 [-0.17, 0.29]

[a] Pro-inflammatory latent factor: IL-1β, IL-6, MCP-1, sFlt-1, CRP
Anti-inflammatory latent factor: IL-2, IFN-γ, TNF-α, IL-4, IL-5, IL-10
[b] Estimated unstandardized regression coefficients and corresponding 95% confidence intervals are shown, after adjustment for the following covariates: maternal age at enrollment, child age at testing, child sex, number of parents living with the child, and Hollingshead socioeconomic status.
MacArthur-Bates CDI: Communicative Development Inventories, Words & Gestures
* $p < .05$.