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Associations between maternal cytokine levels during gestation and measures of child cognitive abilities and executive functioning

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ABSTRACT

Preclinical studies demonstrate that environmentally-induced alterations in inflammatory cytokines generated by the maternal and fetal immune system can significantly impact fetal brain development. Yet, the relationship between maternal cytokines during gestation and later cognitive ability and executive function remains understudied. Children (n = 246) were born of mothers enrolled in the Newborn Epigenetic Study – a prospective pre-birth cohort in the Southeastern US. We characterized seven cytokines [$IL-1\beta$, $IL-4$, $IL-6$, $IL-12p70$, $IL-17A$, tumor necrosis factor-α ($TNF\alpha$), and interferon-γ ($IFN\gamma$)] and one chemokine ($IL-8$) from maternal plasma collected during pregnancy. We assessed children's cognitive abilities and executive functioning at a mean age of 4.5 (SD = 1.1) years. Children's DAS-II and NIH toolbox scores were regressed on cytokines and the chemokine, controlling for maternal age, race, education, body mass index, IQ, parity, smoking status, delivery type, gestational weeks, and child birth weight and sex. Higher IL-12p70 ($\beta_{IL-12p70}$ = 4.26, p = 0.023) and IL-17A (β_{IL-17A} = 3.70, p = 0.042) levels were related to higher DAS-II GCA score, whereas higher IL-1β (β_{IL-1B} = −6.07, p = 0.003) was related to lower GCA score. Higher IL-12p70 was related to higher performance on NIH toolbox measures of executive functions related to inhibitory control and attention ($\beta_{IL-12p70}$ = 5.20, p = 0.046) and cognitive flexibility ($\beta_{IL-12p70}$ = 5.10, p = 0.047). Results suggest that dysregulation in gestational immune activity are associated with child cognitive ability and executive functioning.

1. Introduction

Neurodevelopmental impairments affect an estimated 15% of children aged 2 to 8 years in the United States (Bitsko et al., 2016). Throughout the life course, childhood neurodevelopmental delays and cognitive impairments extend to poor academic achievement, higher engagement in health risk behaviors, increased risk for chronic disease and mental health problems, and lack of economic productivity in adulthood (Engle et al., 2007; Walker et al., 2011; Heckman, 2008; Heckman and Raut, 2016; Shonkoff, 2016). Previous studies indicate that both genetic factors (Sniekers et al., 2017; Gialluisi et al., 2014; Hansell et al., 2015) and the prenatal milieu profoundly affect brain development and neurodevelopmental abilities later in childhood (Park et al., 2016; von Ehrenstein et al., 2012; Singer et al., 2016). A better understanding of the prenatal factors contributing to suboptimal cognitive abilities and neurodevelopmental outcomes could be useful to developing prevention strategies to promote optimal development in these areas.

Environmental exposures, such as toxins, stress, nutrition, and infection during pregnancy have all been linked with behavioral, cognitive and neurodevelopmental impairments in offspring (Lee et al., 2016). Emerging evidence suggests that some of these exposures exert their influence via maternal immune regulation (Calderon-Garciduenas et al., 2009; Wright et al., 2010; Gilman et al., 2017). Several immune molecules produced in response to microglial activation within the central nervous system (CNS) are

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implicated in neurogenesis, synaptic maturation, generation of neural networks and other important processes of brain development (McAfoose and Baune, 2009; Bilbo and Schwarz, 2009). For example, cytokines are common immune and neuronal cell signaling proteins, with the ability to pass through the blood-brain barrier directly through active transport mechanisms, or indirectly through other means. Thus, if maternal cytokines mediate bidirectional brain-immune communication across the placental and blood-brain barriers during fetal development, it is possible that they also influence measurable differences in children's cognitive and neurodevelopmental abilities (von Ehrenstein et al., 2012). Determining the degree to which gestational immune activity relates to cognitive ability and executive functioning in children could help elucidate potential pathways by which the prenatal environment influences brain development, cognitive and neurodevelopmental abilities.

There is a growing, yet limited, set of studies that have investigated the role of gestational immune markers and subsequent neurodevelopmental outcomes in children (McAfoose and Baune, 2009; Hohlfeld et al., 2007). Previously conducted studies using cell cultures and model organisms have already demonstrated the critical role that prenatal circulating cytokines and chemokines play in brain development (Deverman and Patterson, 2009). In humans, developmental neuropsychiatric conditions, such as schizophrenia in adulthood and autism in childhood, have been linked with elevated gestational levels of cytokines and chemokines (Brown et al., 2004; Jones et al., 2017). Further, lower gestational levels of IL-8 have been associated with physician assessed neurological abnormalities at 1 year of age (Gilman et al., 2017) , and at least one study has shown that umbilical cord blood concentrations of inflammatory cytokines are associated with child cognitive intellectual abilities (i.e., intellectual quotient (IQ)) (von Ehrenstein et al., 2012). These studies highlight the association between gestational immune activity and broad-based cognitive abilities, but less is known regarding the extent to which gestational immune activity relates to other domains of functioning, such as those related to executive functioning. Executive functions are neurocognitive processes involved with the execution of goal directed behaviors and optimization of cognitive resources in contexts with competing or unexpected demands. Deficits in executive functions are related to a number of childhood neurodevelopmental problems (Rosenthal et al., 2013; Snyder et al., 2015; Willcutt et al., 2005). Yet, no study to date has examined the extent to which gestational immune activity specifically relates to executive functioning.

The present study contributes novel features to the growing body of literature examining gestational cytokines in relation to child cognitive and neurodevelopment in a variety of ways. First, while previous studies have examined broad-based neurodevelopmental outcomes, and one study has examined intellectual ability (IQ) as an outcome (Mansouri et al., 2009), this study includes assessment of both cognitive ability and executive functioning. This allows for the extension of previous research on intellectual abilities, while gaining further insight into the specific cognitive domains that may be linked to gestational immune functioning. Second, we included in our assessment the significance and direction of the effect of seven cytokines $IL-1\beta$, $IL-4, IL-6$, $IL-12p70$, $IL-17A$, tumor necrosis factor-α ($TNF\alpha$), and interferon-γ ($IFN\gamma$)] and one chemokine ($IL-8$). This panel was custom built based on existing pre-clinical and clinical literature (i.e., analytes with a putative role in normal neurodevelopment (Bilbo and Schwarz, 2009), previously associated with either neuroprotection or adverse neurodevelopmental outcomes (e.g., IL-1β, IL - 6) in model organism or human studies (von Ehrenstein et al., 2012; Gilman et al., 2017; Bilbo and Schwarz, 2009; Jones et al., 2017), while balancing feasibility in number of analytes assessed. Finally, we included a number of covariates in our models, including the assessment of maternal cognitive ability and executive function, thereby enabling the assessment of the magnitude of the effect of gestational cytokines and chemokines beyond the potential genetic or environmental contributions of maternal cognitive ability and executive functioning.

2. Materials and methods

2.1. Participants

Participants were part of the Newborn Epigenetic Study (NEST), a Southeastern United States pre-birth cohort initiated in 2005. The Institutional Review Board approved studies involving these participants, and informed written consent was obtained from all participants. Participant identification and enrollment procedures are described elsewhere (Liu et al., 2012; Hoyo et al., 2011). Briefly, 2595 pregnant women were recruited from prenatal clinics serving Duke University Hospital and Durham Regional Hospital Obstetrics facilities from April 2005 to June 2011. Eligibility criteria were as follows: aged $\geq$ 18 years, pregnant, and had intentions to use one of the two obstetrics facilities enabling access to labor and birth outcome data for the index pregnancy. Maternal blood specimens were collected along with survey data on health, nutrition, stress, and lifestyle behaviors during the enrollment period, which occurred during the first trimester for most participants.

In 2014, women with live births and who had agreed to be recontacted were recruited for a follow-up study examining prenatal smoke exposure, epigenetics, childhood Attention Deficit Hyperactivity Disorder (ADHD) symptoms, cognitive abilities, and executive functioning development. Eligibility criteria required women participants to speak English and have had stored biological samples collected during the prenatal phase and at birth (maternal plasma samples and cord blood samples). Analyses were conducted on 246 mother-child pairs from this study for whom we had cytokine data already assayed and for whom we had assessments of child cognitive ability and executive functioning. Children had to be at least 3 years of age to allow for executive functioning testing. Compared to the overall NEST sample, the analysis sample included a higher percentage of women who were college graduates (41.3% vs 32.3%, $\chi^2 = 9.70$, $p = 0.02$) and a greater percentage of women who were African American (58.5% vs 42.0%, $\chi^2 = 45.13$, $p < 0.001$). There were no differences with respect to factors potentially related to gestational immune functioning: self-reported smoking status in the analysis group was generally similar to the full sample (19.1% vs 24.6%, $\chi^2 = 3.66$, $p = 0.06$), Cesarean delivery was similar (34.4% vs 37.9%, $\chi^2 = 2.33$, $p = 0.31$), prevalence of gestational diabetes was similar (6.7% vs 7.1%, $\chi^2 = 4.83$, $p = 0.18$), and the mean pre-pregnancy Body Mass Index (BMI) was similar (28.7 vs 28.0, $p = 0.24$).

2.2. Assessment of child cognitive ability and executive functioning

Eligible mothers in the larger NEST cohort were contacted via recruitment letters mailed from the study team and/or were recruited during a well-child clinical visit. Mothers who agreed to participate were scheduled for a three-hour visit at study clinic offices where they provided informed consent, were asked to complete survey measures on their child's health and behaviors, and were administered assessments for cognitive ability and executive functioning. Meanwhile, another staff member administered assessments for cognitive ability and executive functioning to their child. A licensed clinical psychologist trained staff in standardized administration of these assessments.

Child cognitive ability was assessed using the Differential Abilities Scale, second edition (DAS-II) (Elliot, 2007). The DAS-II yields a

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