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Are there early inflammatory biomarkers that affect neurodevelopment in infancy?☆

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ABSTRACT

Few studies have investigated the relationship between post-natal inflammatory biomarkers at early age and child neurodevelopment outcomes. The main aim of this study was to examine the relationship between IL-6, IL-1β, IL-4 cytokines, as well as cortisol at 6 and 12 months of age, and neurodevelopment and psychological problems at 30 months of age.

The study was conducted on a sample of 51 full-term newborns who were followed up at 6, 12, and 30 months of age. Infant neurodevelopment was assessed using the *Bayley Scales of Infant Development-II*, psychological problems were assessed with the *Child Behavior Checklist 1.5–5* (CBCL 1.5–5) and the mother's emotional symptoms were assessed with the *General Health Questionnaire-28*. When the infants were 6 and 12 months old, IL-6, IL-1β, IL-4 cytokines, and cortisol were measured in blood samples. The results showed that higher IL-6 at 12 months predicted higher scores in internalizing (emotionally reactive, anxious/depressed, withdrawn and attention problems) and externalizing problems (aggressive behavior) at 30 months. By contrast, high levels of IL-1β at 6 months were related to worse motor skills. Inflammatory biomarkers were not related to mental performance. IL-6 and IL-1β could be early markers of later psychological problems and psychomotor disabilities.

1. Introduction

There is an increasing interest in studying the relationship between inflammation biomarkers and children's neurodevelopmental outcomes (Jiang et al., 2014; Krakowiak et al., in press). Inflammation is known to be a natural defense mechanism by body tissues in response to the recognition of injury, but this process may stop being protective for the organism and become harmful when it occurs chronically (Esteban-Cornejo et al., 2016; Tyrka et al., 2015). Consequently, the inflammation processes may have important negative effects on neural plasticity and neurogenesis, and may also be related to gray matter atrophy as has been shown in animal models (Yirmiya & Goshen, 2011). Within the multiple range of inflammatory biomarkers that have been examined in the context of possible relationships with mental disorders, the most commonly measured are cytokines (Baumeister et al., 2014). There are pro-inflammatory cytokines, which are involved in the up-regulations of inflammatory reactions, and anti-inflammatory cytokines which control the pro-inflammatory cytokine response.

To our knowledge, few studies have examined the possible link between children's inflammatory biomarkers and their neurodevelopment and mental health, and most of these studies were conducted with samples of extremely premature infants or with clinical samples of children with autism spectrum disorders (ASD) (Businaro et al., 2016; Carlo et al., 2011; Leviton et al., 2016; Masi et al., 2015; Ricci et al., 2013). Furthermore, most of these studies have focused on the relationship between mothers' inflammatory cytokines during pregnancy and their children's neurodevelopmental outcomes, or the presence of mental illness disorders later in life (Ratnayake et al., 2013). In this regard, Jiang et al. (2014) stated that no studies have linked markers of inflammation during the post-neonatal period to child development. Those authors found that inflammation biomarkers in the first year of life were related to neurodevelopment outcomes in infants living in conditions of poverty. In specific terms, elevated serum levels of pro-inflammatory cytokines (IL-1β and IL-6) were associated with poor scores for motor skills, whereas elevated serum levels

☆ Appendix: DeFensas research team, Núria Aranda, Victoria Arija, Josep Maria Barroso, Cristina Bedmar, Josefa Canals, Joaquín Escribano, Carmen Hernández-Martínez, Cristina Jardí, Rosa Jiménez, Blanca Ribot, and Núria Voltas.

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of the T helper 2 (Th2) cytokine IL-4 were associated with elevated scores for cognitive skills at 12 and 24 months. Likewise, Tyrka et al. (2015) found that IL-1β was associated with adverse experiences such as child maltreatment or socioeconomic adversity and was involved in the neuropathology of psychiatric conditions in early childhood. Moreover, Pandey et al. (2012), using a sample of teenage suicide victims found high levels of IL-1β, IL-6, and TNF-α in the prefrontal cortex, thus linking cytokine levels with mood disorders, another issue that has also been widely investigated. Other studies have therefore corroborated that pro-inflammatory cytokines may be part of the pathophysiology of emotional disorders; in particular adolescent females with clinical anxiety and/or depression showed high plasma levels of IL-2, IL-1β, and IL-10 (Henje Blom et al., 2012). Furthermore, a study of patients between 8 and 17 years old who presented affective, anxiety, psychotic, obsessive-compulsive, tic or tourette's disorders showed that they presented higher levels of IL-1β, IL-6, IL-8, IFN-γ-induced protein-10 (IP-10), Monocyte Chemoattractant Protein-1 (MCP-1) and monocytes (Gariup et al., 2015). Meanwhile, in a community sample of Taiwanese children, the results showed that IL-1β was positively associated with anxiety and depression symptoms (Chung et al., 2014). In a case-control study, Yang et al. (2015) indicated that cortisol, IL-6 and TNF-α also had an association with ASD severity. In particular, subjects with ASD showed lower levels of cortisol, and high levels of IL-6 and TNF-α. Despite these studies, there is a lack of data from pediatric community samples.

Similarly, studies with adolescent and adult samples have also shown associations between alterations in serum levels of cytokines and the presence of schizophrenia, major depression, post-traumatic stress disorder and bipolar disorder (Blom et al., 2012; Gola et al., 2013; Goldstein et al., 2015; Müller et al., 2015). Despite some controversial results, there therefore appears to be a relation between certain biomarkers and several psychiatric disorders, but further research is needed to identify the pathways that explain this relationship.

In the existing literature based on animal models and human subjects, few studies have investigated the influence of inflammatory markers on infant neurodevelopment. The main aim of this study was therefore to test the possible association between certain inflammatory biomarkers [IL-6, IL-1β, and IL-4 cytokines] and cortisol (which affects the immune response) measured at 6 and 12 months of age, and neurodevelopment and psychological problems at 30 months of age. We hypothesized that some of these early biomarkers may influence neurodevelopment and the presence of psychopathology.

2. Materials and methods

2.1. Procedure

The study was approved by the Research and Ethics Committee of Sant Joan University Hospital in Reus (Spain) and informed consent was obtained from all the participants. The sample was recruited at Sant Joan University Hospital between 2006 and 2009. The inclusion criteria for mothers were that they should be pregnant with no >11 weeks of gestation and over 18 years old, and the exclusion criteria were presenting with a chronic illness affecting nutritional status such as diabetes type I, Crohn's or celiac diseases, having a multiple pregnancy or having gone to another hospital to give birth. Meanwhile, the inclusion criteria for infants were that they should have a gestational age ≥37 weeks, be Caucasian, be from families that understand Catalan or Spanish, and have no medical problems; the exclusion criteria were birth weight <2500 g, the presence of an illness associated with iron metabolism, birth defects, immunodeficiency or hypothyroidism, and/or diseases requiring intensive care.

Socio-demographic, anthropometric, nutritional, psychopathological data, and blood samples were collected from these infants during the study phases. Data were also collected on the mothers' toxic habits during pregnancy (smoking, alcohol and/or drug consumption) and birth data. The follow-up study consisted of three visits: when the infants were 6 months, 12 months, and 30 months old (see the flow diagram of the study in Fig. 1).

2.2. Participants

An initial sample of 158 newborns belonging to a larger project studying the effect of nutrition on psychological development was followed up at 6, 12, and 30 months. Of these, 51 infants completed all the psychological and biomedical assessments (25 boys) until 30 months of age. Thus, the follow-up sample contained 51 infants and the final statistical power was 98% according to the means for the total problems CBCL score and the distribution in IL groups.

The mean weight at birth was 3241.4 g (SD = 471.7), the gestational mean age was 39.6 weeks (SD = 1.2), and their mothers had a mean age of 32.4 years (SD = 4.6). As regards the socioeconomic level of the families, 88.3% were middle-high class. Apgar scores were 8.8 at 1 min (SD = 1.0) and 9.9 at 5 min (SD = 0.3). None of the pregnant women had consumed alcohol or illicit drugs during pregnancy, but 21.6% smoked during pregnancy (11.8% smoked between 1 and 5 cigarettes per day, 3.9% between 6 and 10 cigarettes per day, and 5.9% >10 cigarettes) and 15.7% of these mothers were secondhand smokers (more data on the sample are shown in Table 1).

2.3. Measures

2.3.1. Biochemical measurements

At 6 and 12 months blood samples were collected by venipuncture into tubes with no anticoagulants to obtain serum early in the morning at Sant Joan University Hospital in Reus. The blood drawings were performed by hospital staff nurses from the Pediatrics service. Blood extractions were programed a few days before performing the child's assessment and interview with parents, or in some cases in the same day. The analyses were performed immediately, or aliquots of serum were stored in the Biobanc of the Institut d'Investigació Sanitària Pere Virgili at −80° for subsequent measurements. IL-6, IL-1β, IL-4 cytokines were measured by FlowCytomix™ Multiplex assay (Bender MedSystems, Vienna, Austria) in accordance with the manufacturer's instructions. The kit allowed simultaneous quantification of three cytokines (IL-6, IL-1β and IL-4) by combining the human single cytokine and the Human Basic FlowCytomix kits. Samples were analyzed in a Coulter Epics XL-MLC flow cytometer (Beckman-Coulter). Concentration of each cytokine was obtained by interpolating fluorescence intensity to a 7-point dilution standard curve supplied by the manufacturer (ranged between 27 and 20,000 pg/mL) using FlowCytomix Pro 2.2 Software (Bender MedSystems). The minimum detectable concentrations of IL-6, IL-1β and IL-4 were 1.2, 4.2 and 20.8 pg/mL, respectively. Any cytokine value below the limits of detection was given zero. Plasma cortisol was measured by new electrochemiluminescence immunoassay technology, namely the Roche Elecsys 2010 Chemistry Analyzer. The lowest detection limits were 0.500 nmol/L. All the biochemical parameters were dichotomized into the two lowest tertiles and the highest tertile. In this sample we also measured ultrasensitive C-reactive protein values by using a CRP immunoturbidimetric reagents assay (Roche diagnostics, Mannheim, Germany) and the detection limits were 0.3 mg/L. The entire sample showed CRP levels in the normal range.

2.3.2. The Child Behavior Checklist 1.5–5

The Child Behavior Checklist 1.5–5 (CBCL 1.5–5; Achenbach & Rescorla, 2000) is a 110-item parent-report questionnaire that assesses behavioral and emotional problems. The CBCL 1.5–5 provides scores for seven scales: emotionally reactive, anxious/depressed, somatic complaints, withdrawn (internalizing problems), attention problems and aggressive behavior (externalizing problems), and sleep problems. The sum of the scores for all the problem items makes up the total psychological problems category. For this study, the mother and the father