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Dietary omega-3 deficiency exacerbates inflammation and reveals spatial memory deficits in mice exposed to lipopolysaccharide during gestation

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ABSTRACT

Maternal immune activation (MIA) is a common environmental insult on the developing brain and represents a risk factor for neurodevelopmental disorders. Animal models of *in utero* inflammation further revealed a causal link between maternal inflammatory activation during pregnancy and behavioural impairment relevant to neurodevelopmental disorders in the offspring. Accumulating evidence point out that proinflammatory cytokines produced both in the maternal and fetal compartments are responsible for social, cognitive and emotional behavioral deficits in the offspring.

Polyunsaturated fatty acids (PUFAs) are essential fatty acids with potent immunomodulatory activities. PUFAs and their bioactive derivatives can promote or inhibit many aspects of the immune and inflammatory response. PUFAs of the n-3 series ('n-3 PUFAs', also known as omega-3) exhibit anti-inflammatory/pro-resolution properties and promote immune functions, while PUFAs of the n-6 series ('n-6 PUFAs' or omega-6) favor pro-inflammatory responses. The present study aimed at providing insight into the effects of n-3 PUFAs on the consequences of MIA on brain development. We hypothesized that a reduction in n-3 PUFAs exacerbates both maternal and fetal inflammatory responses to MIA and later-life defects in memory in the offspring.

Based on a lipopolysaccharide (LPS) model of MIA (LPS injection at embryonic day 17), we showed that n-3 PUFA deficiency 1) alters fatty acid composition of the fetal and adult offspring brain; 2) exacerbates maternal and fetal inflammatory processes with no significant alteration of microglia phenotype, and 3) induces spatial memory deficits in the adult offspring. We also showed a strong negative correlation between brain content in n-3 PUFA and cytokine production in MIA-exposed fetuses. Overall, our study is the first to address the deleterious effects of n-3 PUFA deficiency on brain lipid composition, inflammation and memory performances in MIA-exposed animals and indicates that it should be considered as a potent environmental risk factor for the apparition of neurodevelopmental disorders.

1. Introduction

Maternal immune activation (MIA), occurring in a context of bacterial or viral infection, is a common environmental insult on the developing brain and represents a risk factor for neurodevelopmental disorders such as schizophrenia, autism or cerebral palsy (Bilbo et al., 2018; Estes and McAllister, 2016; Fleiss and Gressens, 2012; Hagberg et al., 2015; Knuesel et al., 2014; Madore et al., 2016; Patterson, 2011, 2009; Van Steenwinckel et al., 2014). MIA has recently been associated to altered connectivity in the prefrontal cortex, temporo-parietal junction, and basal ganglia of neonates and toddlers, further linking prenatal inflammation to psychiatric risk in humans (Spann et al., 2018).

Animal models of *in utero* inflammation (triggered by viral polyinosinic:polycytidylic [poly(I:C)] or bacterial [lipopolysaccharide, LPS]

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mimics) further revealed a causal link between maternal inflammatory activation during pregnancy, disorganized brain cytoarchitecture and behavioural impairment relevant to neurodevelopmental disorders in the offspring (Bilbo and Schwarz, 2012; Choi et al., 2016; Deverman and Patterson, 2009; Estes and McAllister, 2016; Fernández de Cossío et al., 2017; Hui et al., 2018; Shin Yim et al., 2017). Accumulating evidence point out that proinflammatory cytokines produced both in the maternal and fetal compartments, are responsible for social, cognitive and emotional behavioral deficits in the offspring (Ashdown et al., 2006; Bilbo et al., 2018; Cai et al., 2000; Estes and McAllister, 2015; Golan et al., 2005; Hao et al., 2010; Liverman et al., 2006; Urakubo et al., 2001). Interleukin-1beta (IL-1β), IL-6 and Tumor Necrosis Factor alpha (TNFα) disrupt brain structures connectivity involved in social, emotional and memory processes (Baharnoori et al., 2009; Cai et al., 2000; Fatemi et al., 2009; Giovanoli et al., 2015; Golan et al., 2005; Graciarena et al., 2010; Lowe et al., 2008; Samuelsson et al., 2006). More recently, IL-17 has been shown to mediate maternal poly-IC-induced social behavior impairment and abnormal cortical development in offspring (Choi et al., 2016; Shin Yim et al., 2017). A particular role has been attributed to maternal and fetal IL-6 in offspring cognitive disorders in humans (Spann et al., 2018). Indeed, prenatal exposure to IL-6 recapitulates the deficits in hippocampal synaptic transmission and spatial learning that are classically observed in adult offspring in a context of MIA (Patterson, 2009; Samuelsson et al., 2006). These deficits can be reversed by treating the mothers with an anti-IL-6 antibody (Mouihate and Mehdawi, 2016). Hence, the production of IL-6 in the pregnant mother and in the fetal brain is critical to MIA-induced cognitive impairment in the offspring.

Polyunsaturated fatty acids (PUFAs) are essential fatty acids with potent immunomodulatory activities (Calder, 2017). PUFAs and their bioactive derivatives can promote or inhibit many aspects of the immune and inflammatory response. Notably, PUFAs of the n-3 series ('n-3 PUFAs', also known as omega-3) exhibit anti-inflammatory/pro-resolutive properties and promote immune functions, while PUFAs of the n-6 series ('n-6 PUFAs' or omega-6) favor pro-inflammatory responses (Calder, 2006, 2001; Layé et al., 2018; Orr et al., 2013b). As vertebrates lack the necessary enzymes for *de novo* synthesis of n-6 and n-3 PUFAs, these fatty acids have to be provided by the diet (Bazinet and Layé, 2014). When increased by dietary or genetic approaches, we and others showed that n-3 PUFAs down-regulate the production of proinflammatory cytokines both at the periphery and in the brain, while n-6 PUFAs promote their synthesis and release (Delpech et al., 2015b, 2015a; Fourrier et al., 2017; Hopperton et al., 2017, 2016; Labrousse et al., 2012; Madore et al., 2014; Mingam et al., 2008; Orr et al., 2013a). The central anti-inflammatory effects of n-3 PUFAs are mainly mediated by docosahexaenoic acid (DHA, 22:6 n-3), the main long chain (LC) n-3 PUFA accumulating in the brain (Bazinet and Layé, 2014; Layé et al., 2018; Orr et al., 2013b). DHA targets microglia, the brain resident innate immune cells to dampen the production and action of the proinflammatory cytokines IL-6, IL-1β and TNFα (De Smedt-Peyrusse et al., 2008; Fourrier et al., 2017; Inoue et al., 2017; Mancera et al., 2017; Nadjar et al., 2016; Tremblay et al., 2016). Conversely, low level of DHA in the brain enhances the production of proinflammatory cytokines and affects microglia phenotype and function, with a polarization of these cells to a pro-inflammatory phenotype (Delpech et al., 2015b; Madore et al., 2014; McNamara et al., 2010; Nadjar et al., 2016).

The maternal dietary status in n-3 PUFAs is crucial for the offspring, as the embryo cannot produce its own DHA and therefore entirely depends on maternal supply (Gibson et al., 1996). DHA is transferred from the mother to the offspring during gestation (blood supply) and lactation (milk) (Hanebutt et al., 2008; Innis, 2005; Lewis et al., 2018). Preterm infants or infants from mothers with dietary deficit in n-3 PUFAs have limited n-3 PUFA stores in the body and brain (Larque et al., 2002; Makrides et al., 1994). This might enhance the immune response and aggravate the cognitive deficits associated with prenatal infection.

The present study aimed at providing insight into the effects of n-3 PUFA dietary intake during pregnancy and lactation on the consequences of MIA on brain development. We hypothesized that a reduction in maternal dietary n-3 PUFA exacerbates both maternal and fetal inflammatory responses to MIA and later-life defects in hippocampal connectivity and related memory behavior in the offspring. To this end, we evaluated developmental changes in offspring brain fatty acid composition as well as proinflammatory cytokine production in response to MIA (LPS at embryonic day 17) in a well-established dietary model of n-3 PUFA deficiency (Lafourcade et al., 2011; Madore et al., 2014; Mingam et al., 2008; Moranis et al., 2012). Long-term memory and hippocampal integrity were evaluated in the adult offspring. Our results revealed that maternal n-3 PUFA deficiency worsens the effects of prenatal LPS on memory performances and brain pro-inflammatory cytokines production in the offspring. We also showed that IL-6 production is strongly anti-correlated to brain DHA levels, emphasizing the crucial role of this fatty acid in the development of later life cognitive abilities.

2. Materials and methods

2.1. Animals

Animal husbandry and experimental procedures were in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the national ethical committee for care and use of animals (approval ID A13169). Every effort was made to minimize suffering and the number of animals used. All experiments were made on C57BL6/J males and females (Charles River, Arbresle, France). Mice were maintained under standard housing conditions on corncob litter in a temperature (23 ± 1 °C) and humidity (40–50%) controlled animal room with a 12 h light/dark cycle (07 h–19 h) and *ad libitum* access to food and water.

2.2. Diet and treatment

N-6 and n-3 LC PUFAs can be biosynthesized from their dietary precursors, respectively linoleic acid (18:2n-6 or LA) and α-linolenic acid (18:3 n-3 or ALA) (Lands et al., 1990). Female C57BL6/J mice were fed with isocaloric diets containing 5% fat with a high (n-3 deficient diet) or low LA/ALA ratio (n-3 balanced diet) across gestation and lactation to modulate n-3/n-6 PUFAs in the offspring (Delpech et al., 2015b; Madore et al., 2014; Mingam et al., 2008; Moranis et al., 2012). When studied at adulthood, the male offspring was kept under the same diet as their dams after weaning, except for data presented in Fig. 1B (reversal experiment in Y-maze) for which offspring were fed with n-3 PUFA balanced diet until weaning and then exposed to n-3 PUFA deficient diet until behavioral assessment.

At G17 (17 days after mating), MIA was triggered by the intraperitoneal (i.p.) administration of LPS (E. Coli 0127:B8, Sigma Inc, St. Louis, MO, USA; 0.12 μg/g mouse/100 μl). The administration of the corresponding volume of saline solution (NaCl 0.9%, "Saline") was used as a control (Golan et al., 2005; Roumier et al., 2008).

We generated 4 cohorts of mice in total. Cohort 1 was used to assess memory performances in adults (57 males from 15 dams). In cohort 2, we quantified fatty acids levels and cytokine production in fetuses and dams (30 dams were used, fetuses from the same litter were pooled for n = 1). Cohort 3 was used for FACS analysis (22 dams were used, fetuses from the same litter were pooled for n = 1). Cohort 4 was used to assess cFos expression and measure fatty acid composition of adult livers and brainstems (40 males from 18 dams were used).

2.3. Assessment of memory performances

Memory tests always took place in the morning (between 8:00AM