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Maternal inflammation induces immune activation of fetal microglia and leads to disrupted microglia immune responses, behavior, and learning performance in adulthood

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ABSTRACT

Maternal inflammation during pregnancy can have detrimental effects on embryonic development that persist during adulthood. However, the underlying mechanisms and insights in the responsible cell types are still largely unknown. Here we report the effect of maternal inflammation on fetal microglia, the innate immune cells of the central nervous system (CNS). In mice, a challenge with LPS during late gestation stages (days 15–16–17) induced a pro-inflammatory response in fetal microglia. Adult whole brain microglia of mice that were exposed to LPS during embryonic development displayed a persistent reduction in pro-inflammatory activation in response to a re-challenge with LPS. In contrast, hippocampal microglia of these mice displayed an increased inflammatory response to an LPS re-challenge. In addition, a reduced expression of brain-derived neurotrophic factor (BDNF) was observed in hippocampal microglia of LPS-offspring. Microglia-derived BDNF has been shown to be important for learning and memory processes. In line with these observations, behavioral- and learning tasks with mice that were exposed to maternal inflammation revealed reduced home cage activity, reduced anxiety and reduced learning performance in a T-maze. These data show that exposure to maternal inflammation during late gestation results in long term changes in microglia responsiveness during adulthood, which is different in nature in hippocampus compared to total brain microglia.

1. Introduction

Microglia are the resident innate immune cells of the brain, and important to maintain or restore tissue homeostasis (Nimmerjahn et al., 2005). Microglia originate from progenitors localized in the embryonic yolk sac early during embryonic development, and colonize the neural epithelium, where they proliferate and mature during embryonic development. Microglia form a self-sustained cell population with, under physiological conditions, very little contribution of bone marrow-derived monocytes (Ginhoux et al., 2013). The microglial population in the mouse brain is replaced however, the exact rate of turnover is unresolved. Where BrdU incorporation experiments indicated that the microglia population is replaced every 100 days (Askew et al., 2017), genetic labelling experiments showed that microglia turnover of with different rates in different brain regions. Hippocampal microglia completely turn over in 15 months and cortical microglia in 41 months (Tay et al., 2017). Regional differences in microglia are also observed in gene expression profiles and in regional differences in age-related changes in gene expression (Grabert et al., 2016). When exposed to pathogens or tissue damage, microglia adopt a more activated state, release cytokines, chemokines, neurotrophic factors, and change in migratory and phagocytic behavior (Kettenmann et al., 2011). Since microglia, together with astrocytes, are the primary source of cytokine release in the brain, these cells are of main importance in basal functioning as well as pathologies of the brain.

Microglia colonize the developing CNS early during mammalian embryonic development, a period wherein maternal inflammation can greatly affect fetal microglia. In this time, the placenta allows selective exchange of necessary factors including nutrients, endocrine factors and antibodies. At the same time, it protects the embryo against potential harmful factors in the maternal circulation. Although the placenta is an effective barrier, it can be disrupted by certain adverse conditions such as maternal inflammation. This may lead to improper placental functioning, which in turn can impair normal embryonic development (Hsiao and Patterson, 2012). Maternal inflammation is a serious risk factor for several neurological pathologies and in humans, prenatal

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inflammation has been identified as a risk factor for developing autism (Meldrum et al., 2013; Patterson, 2011), schizophrenia (Patterson, 2009) and cerebral palsy (Yoon et al., 2000). Several animal models have been developed to study this relationship between maternal inflammation and development of these diseases. For example, to mimic a viral infection pregnant animals are treated with polyinosinic:polycytidylic acid (poly-I:C), or to mimic a bacterial infection they are treated with lipopolysaccharide (LPS), a cell wall component of gram negative bacteria. Although these models both induce maternal inflammation and fetal brain inflammation, the physiological consequences of LPS and poly-I:C exposure are different in nature. Where poly-I:C is reported not to affect time of birth and survival of pups, both i.p. and i.v. injection of high dose LPS in pregnant mice had a detrimental effect on both the litter size and survival of the offspring (Arsenault et al., 2013). These observations emphasize that maternal inflammation is not a uniform phenomenon and that potential consequences for the offspring depend on the inflammatory stimulus.

We previously showed that i.p. injection of LPS in adult mice caused a long-term reduction in the pro-inflammatory response to a second LPS challenge in microglia (Schaafsma et al., 2015). Persistent changes induced in these cells by prenatal inflammation might have possible long-term effects in the offspring. Regarding LPS-induced prenatal inflammation, most data were generated using total brain and hippocampus tissue and hence cell type-specific information regarding (cytokine) gene expression and their effects is limited. Furthermore, the effect of LPS-induced maternal inflammation on fetal brain has only been assessed in total fetal brain, where increased levels of pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6 in the mother as well as in the fetal brain were observed during pregnancy (Elovitz et al., 2011; Liverman et al., 2006). Activation of fetal microglia specifically has been shown in poly-I:C induced inflammation (Pratt et al., 2013). However, it is unknown whether LPS-induced fetal brain inflammation can be attributed to maternal factors, or that fetal microglia themselves are immune activated by LPS, and moreover if this results in an altered response to LPS by these microglia during adulthood. Detrimental effects of prenatal inflammation on behavior, structural changes in the brain, and neurogenesis are reported (Boksa, 2010; Chlodzinska et al., 2011; Graciarena et al., 2010; Hao et al., 2010; Lin et al., 2012). In addition, prenatal inflammation has neuroprotective effects against ischemic events (Wang et al., 2007), which is linked to a more tolerant microglia phenotype with reduced expression of pro-inflammatory cytokines (Halder et al., 2013; Rosenzweig et al., 2004). Impaired learning and memory as a consequence of prenatal inflammation is related to increased pro-inflammatory gene expression, mainly IL-1β in the hippocampus (Bilbo and Schwarz, 2009; Williamson et al., 2011). The effect of prenatal inflammation seems to depend on the gestation time at the moment of inflammation, the brain regions analyzed and parameter tested. In the study described here, the effect of prenatal inflammation, induced by injection of LPS during late gestation (GD 15–16–17), was investigated in mice with a focus on microglia in fetal and adult brain, and its effects on behavior and learning performance of these mice during adulthood. Regarding learning and memory impairment, it was shown that microglia-derived BDNF is important for hippocampus-dependent learning tasks (Parkhurst et al., 2013). Here, the effect of prenatal inflammation on BDNF expression in total brain and hippocampal microglia was determined.

2. Materials and methods

2.1. Animals

Wild-type pregnant C57Bl/6JOlaHsd mice (E12) were purchased from Harlan (Harlan, Horst, the Netherlands). Animals were housed under normal conditions in a 12 h:12 h light dark cycle at the central animal facility or in the animal facility of the Center for Lifesciences of the University of Groningen. Pregnant animals were housed individually as well as the offspring after weaning and their first treatment. Food and water were available ad libitum throughout the experiments, except during the T maze learning task (see below). Basal home cage activity of offspring mice was measured using a passive infrared monitoring system and analyzed using ACTOVIEW for excel programmed by Dr. C. Mulder (freely available on request).

2.2. Animal treatments

To avoid batch variations in experiments of different LPS lot numbers, one batch of LPS was used in all experiments (*E. coli* 0111:B4, Sigma-Aldrich, Cat# L4391). Prenatal inflammation was induced in pregnant mice by 3 injections with LPS (0.25, 0.10, and 0.05 mg/kg) at GD 15–16–17, control animals were injected with PBS. Young adult offspring (2–4 months) were injected with either PBS or LPS (0.25 mg/kg) and microglia were isolated 3 h post-injection.

2.3. Open field test

To assess whether prenatal inflammation affected the offsprings' explorative activity in a novel environment, the mice were exposed to a so called open field test. Mice were placed in an open field arena with a diameter of 85 cm and height of 30 cm. The arena was subdivided in a center zone and border zone. Mice were introduced in the arena at the outer wall, were allowed to explored the arena for 5 min and were then returned to their home cage. Between sessions the arena was cleaned with 30% ethanol for removal of olfactory cues. The movement of the animals was tracked using ethovision XT videotracking software (Noldus, The Netherlands).

2.4. Elevated plus maze

To assess whether maternal LPS injection affected anxiety levels in the offspring, the mice were subjected to an elevated plus maze test, a commonly used and well-validated anxiety test in rodents. The elevated plus maze consisted of four 90° angle arms with a center region (5 cm × 5 cm), two open arms (5 cm × 29 cm) and two closed arms (5 cm × 29 cm × 16 cm) and the maze was elevated 80 cm above the floor. The experiments were performed in a separate experimental room during the light phase under bright light conditions (100 lx). Mice were placed in the center region, allowed to explore the plus maze for 5 min, and videotaped for later analysis. In between testing animals, the plus maze was cleaned with 30% ethanol to eliminate olfactory cues. The recordings were analyzed for number of entries into open and closed arms and time spent in open and closed arms. An entry was scored when all 4 paws were in the arm. Entries into open and closed arms and time spent into open and closed arms were expressed as a percentage of total entries and total time spent in the open and closed arms.

2.5. T-maze

To test whether maternal LPS injection affected cognitive performance of the offspring later in life, the offspring mice were subjected to a T maze learning task. The T-maze consisted of 3 tubular, transparent Plexiglas arms (diameter 5 cm, length 27,5 cm): a start arm connected to a start box and two test arms at a 90° angle (left and right test arm). One of the test arms was baited with a food reward consisting of a small crumb of the regular food (0.05–0.1 g). Food crumbs were also placed below perforations at the end of the other test arm to prevent mice from discriminating between baited and non-baited arms by olfactory cues. A 1 cm high rim, 4 cm before the end of the tests arms prevented visual inspection for food presence from a distance. A guillotine door located halfway each arm could be operated manually from the experimenter's position and was used to allow animals only one choice in each training trial. Once the animals chose one arm, the