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Schizophrenia associated sensory gating deficits develop after adolescent microglia activation

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ABSTRACT

Maternal infection during pregnancy is a well-established risk factor for schizophrenia in the adult offspring. Consistently, prenatal Poly(I:C) treatment in mice has been validated to model behavioral and neurodevelopmental abnormalities associated with schizophrenia. By using the Poly(I:C) BALB/c mouse model, we investigated the functional profile of microglia by flow cytometry in relation to progressive behavioral changes from adolescence to adulthood.

Prenatal Poly(I:C) treatment induced the expected sensory gating deficits (pre-pulse inhibition (PPI) of the acoustic startle response) in 100 day-old adult offspring, but only in female not in male descendants. No PPI-deficits were present in 30 day-old adolescent mice. Sensory gating deficits in adult females were preceded by a strong M1-type microglia polarization pattern during puberty as determined by flow cytometric analysis of multiple pro- and anti-inflammatory surface markers. Microglia activation in females did not persist until adulthood and was absent in behaviorally unaffected male descendants. Further, the specific activation pattern of microglia was not mirrored by a similar activation of peripheral immune cells. We conclude that prenatal Poly(I:C) treatment induces post pubertal deficits in sensory gating which are specifically preceded by a pro-inflammatory activation pattern of microglia during puberty.

1. Introduction

Epidemiological studies demonstrated a significant link between systemic maternal immune activation during pregnancy and a higher incidence of schizophrenic disorders in the adult offspring. Children of mothers, who were infected with human influenza virus during their second trimester, showed an increased risk to develop schizophrenia in later life (Brown et al., 2004). Similar results were observed after prenatal infections caused by other viral pathogens (Brown et al., 2004; Mednick et al., 1988), and also after infections caused by bacteria or even parasites like *Toxoplasma gondii* (Sørensen et al., 2009; Mortensen et al., 2007; Brown et al., 2005). Therefore, it is not a specific pathogen but rather the systemic maternal immune activation that seems to be the key factor for enhancing the risk of schizophrenia.

These observations led to the establishment of rodent animal models that are using maternal immune activation during pregnancy by either infectious or non-infectious compounds. Indeed, prenatally challenged offspring developed behavioral and neurochemical abnormalities that resemble those observed in schizophrenic and psychosis-prone individuals. Maternal infection with human influenza virus as well as stimulation with polyinosinic: polycytidylic acid (Poly(I:C)), a synthetic analogue of viral double stranded RNA acting via toll-like receptor (TLR)-3, induces deficits in pre-pulse inhibition (PPI) of the acoustic startle reflex, deficits in latent inhibition (LI), and a higher sensitivity to systemic challenge with amphetamine and NMDA receptor antagonists, which can all be linked to an altered sensitivity in the dopaminergic system (Meyer et al., 2005). Descendants further exhibited behavioral deficits in social interaction (Shi et al., 2003) as well as cognitive deficits in the Morris Water Maze (Meyer et al., 2005, 2008). Interestingly, some of these abnormalities, such as PPI- and LI-deficits, were not detectable before the end of puberty similar to the onset of first schizophrenic episodes in young human adults

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(Meyer et al., 2005, 2006; Meyer and Feldon, 2012). Similar, rat MRI studies on Poly(I:C) descendants showed the development of enlarged lateral ventricles and reduced hippocampal volume over puberty (Piontkewitz et al., 2009), again frequent observations in schizophrenic patients. In summary, these findings point to prenatally induced neurodevelopmental alterations resulting in progressive brain abnormalities over time. These events might be initiated by inflammatory cytokines in the fetal brain, which have been shown to significantly interfere with neurodevelopmental processes (Meyer et al., 2005) and might lead to cytoarchitectural disorganization of neuron-populations (Weinberger, 1995). In addition, prenatal immune challenge also influenced the expression of genes specifically associated with schizophrenia (Fatemi et al., 2008).

However, in humans but also in the Poly(I:C) model, the time around puberty seems to be another critical period for further progression of brain structure abnormalities as well as for the manifestation of schizophrenia related behavioral symptoms. In a preceding study, we could demonstrate that the number of microglial cells in prenatally (gestational day (GD) 9) Poly(I:C) challenged mice was significantly increased during puberty (postnatal day (PND) 30), but not in younger (PND 10) or adult mice (PND 100) (Manitz et al., 2013; Juckel et al., 2011). In adult mice, an increased number of microglial cells persisted only in the frontal association cortex, an area with relevance for schizophrenia. In 30 day-old adolescent mice, microglial cells further showed a significant reduction of cell processes, indicating either some kind of activation or dysfunction of these cells (Manitz et al., 2013; Juckel et al., 2011).

In the developing brain, microglia act as amoeboid phagocytes which participate in generating the neuronal network by eliminating aberrant cells and dysfunctional synaptic connections (van Rossum and Hanisch, 2004; Schaefer et al., 2012). Gestational immune activation might result in activation of fetal microglia with release of cytokines or neurotoxic substances thereby interfering with neurodevelopmental processes. In addition, it might also provoke functional long-term effects in these cells with altered reaction patterns mediated e.g. by epigenetic changes. Further, an activation of microglia leads to a functional shaping in case of subsequent activation (Biswas and Mantovani, 2010; Bowdish et al., 2007), which is verifiable over days to weeks after the initial event. The formation of functional microglia subsets known as M1 or M2 could be seen as such an adaptive process (Bowdish et al., 2007). M1 cells (induced by IFN-γ) are characterized by a pro-inflammatory phenotype and secretion of neurotoxic substances (e.g., NO, glutamate), while M2 cells (induced by IL-4, IL-13 or TGF-β) are characterized by an anti-inflammatory phenotype and secretion of neurotrophic factors (e.g., BDNF) (Mantovani, 2008; Koning et al., 2009). Further, M2 cells can be subdivided into M2a, M2b and M2c phenotypes (Chhor et al., 2013). M2a cells, which are induced by IL-4 and IL-13, are involved in tissue repair or the secretion of neurotrophic factors like BDNF and characterized by an anti-inflammatory phenotype (Mantovani, 2008; Koning et al., 2009). IL-1 receptor ligands, immune complexes and toll-like receptor agonists induce M2b cells which rather function similar to M1 cells by exerting immunomodulatory effects. It has been shown that M1/M2b cells induce increasing neuronal loss (Chhor et al., 2013). M2c cells are characterized by an acquired deactivating phenotype. They are induced by TGF-β, glucocorticoids, and IL-10 (Chhor et al., 2013). The classification of microglial phenotypes differs across literature. A useful concept in understanding the functional state of microglia is the M1 and M2 classification, which describes two opposite activation states (Hu et al., 2015). Thus, prenatal immune activation might cause persistent functional changes in microglia that will interact with subsequent environmental challenges in the offspring such as infections, hormonal alterations, and emotional pressure or stress, all known to be able to activate microglial cells. Due to their neurotoxic properties, activated microglia could lead to sustained neuropil reduction with a diminution of synapses, dendrites and spines during the progression of illness (Radewicz et al., 2000).

In the present study, we were interested in the mechanisms underlying progressive changes in prenatally Poly(I:C) challenged offspring finally leading to a schizophrenia-like behavioral phenotype. In this context, we aimed to characterize the functional profile of microglia that were isolated from complete brains by their expression levels of functional surface molecules by flow cytometry.

2. Methods and materials

2.1. Animals

Female and male BALB/c mice were obtained from Charles River (Sulzfeld, Germany) and maintained under standard laboratory conditions for a minimum of 2 weeks to habituate. Mice were mated overnight, and the presence of a vaginal plug marked that day as gestational day (GD) 0. The experimental procedures were approved by the local animal research review committee and were performed according to institutional guidelines.

2.2. Prenatal treatment

At GD 9 pregnant mice were injected with Poly(I:C) (20 mg/kg) intraperitoneally. Control mice were given injections of sterile sodium chloride (0.9%). After treatment, pregnant mice were double-housed until birth. Descendants were weaned and sexed on postnatal day (PND) 28. Males and females were caged separately and littermates of the same sex were housed in groups of 2–4 animals per cage. Experimental groups of vehicle and Poly(I:C) descendants were composed from multiple independent litters to prevent litter effects at PND 30 (adolescence) and PND 100 (adulthood). All litters consisted of both sexes.

2.3. Maternal cytokine analysis

The effect of prenatal treatment on the maternal cytokine response was analyzed with the BD Cytometric Bead Array (CBA) Mouse Enhanced Sensitivity Flex Sets (BD Bioscience) according to the manufacturer's instructions. Blood from pregnant mothers was taken 2 h after intraperitoneal injection of 20 mg/kg Poly(I:C). IL-1β, IL-6, and TNF-α were analyzed in unstimulated heparinized blood samples and were measured on a FACSCanto II (BD Biosciences) and analyzed by FCAP Array software v3 (Soft Flow).

2.4. PPI

For the detection of sensory gating deficits, we measured the pre-pulse inhibition (PPI) of the acoustic startle response adapted to previous protocols (Ibi et al., 2009; Arai et al., 2008). A small restrainer with the mouse was placed into a sound-attenuated chamber using a piezoelectric measuring platform (Startle Response System, TSE, Germany) recording the animaĺs movement. After a habituation time of 5 min, using baseline background noise of 65 dB (white noise), test sessions consisted of three different trial types: a) startle pulse alone (120 dB white noise, 20 ms); b) no stimulus (65 dB white noise); c) pre-pulse (90 dB) followed by the startle pulse (120 dB) with an interstimulus interval of 100 ms. After a presentation of five pulse alone trials, a total of ten presentations of each type were given in a randomized order