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Minocycline rescues decrease in neurogenesis, increase in microglia cytokines and deficits in sensorimotor gating in an animal model of schizophrenia

Daniele Mattei [a,1], Anaïs Djodari-Irani [a,c,1], Ravit Hadar [b], Andreas Pelz [a], Lourdes Fernandez de Cossío [a], Thomas Goetz [b], Marina Matyash [a], Helmut Kettenmann [a], Christine Winter [b,1], Susanne A. Wolf [a,*,1]

[a] Max-Delbrück-Center of Molecular Medicine, Cellular Neuroscience, 13125 Berlin, Germany
[b] University Hospital, Clinic for Psychiatry and Psychotherapy, Experimental Psychiatry, 01307 Dresden, Germany
[c] Department of Psychiatry and Psychotherapy, Charité – Universitätsmedizin Berlin, Charité Campus Mitte, 10117 Berlin, Germany

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ABSTRACT

Adult neurogenesis in the hippocampus is impaired in schizophrenic patients and in an animal model of schizophrenia. Amongst a plethora of regulators, the immune system has been shown repeatedly to strongly modulate neurogenesis under physiological and pathological conditions. It is well accepted, that schizophrenic patients have an aberrant peripheral immune status, which is also reflected in the animal model. The microglia as the intrinsic immune competent cells of the brain have recently come into focus as possible therapeutic targets in schizophrenia.

We here used a maternal immune stimulation rodent model of schizophrenia in which polyinosinic–polycytidilic acid (Poly I:C) was injected into pregnant rats to mimic an anti-viral immune response. We identified microglia IL-1β and TNF-α increase constituting the factors correlating best with decreases in net-neurogenesis and impairment in pre-pulse inhibition of a startle response in the Poly I:C model. Treatment with the antibiotic minocycline (3 mg/kg/day) normalized microglial cytokine production in the hippocampus and rescued neurogenesis and behavior. We could also show that enhanced microglial TNF-α and IL-1β production in the hippocampus was accompanied by a decrease in the pro-proliferative TNFR2 receptor expression on neuronal progenitor cells, which could be attenuated by minocycline. These findings strongly support the idea to use anti-inflammatory drugs to target microglia activation as an adjunctive therapy in schizophrenic patients.

1. Introduction

Schizophrenia is a devastating disorder and constitutes a social and economic burden for patients as well as families and society. During the past decades, an increasing number of studies have associated schizophrenia and inflammation (Müller and Schwarz, 2010; Fineberg and Ellman, 2013). Concomitantly, microglia cells – the intrinsic immune competent cells of the brain – have been pinpointed in the pathophysiology of this neurodevelopmental disorder in both human patients and animal models of this disorder (Blank and Prinz, 2013; Fricker et al., 2013; Harry and Kraft, 2012). In a subpopulation of schizophrenic patients increased microglial cellular density and activity has been found in post-mortem tissue and *in vivo* (Falkai et al., 1999; Steiner et al., 2006; van Berckel et al., 2008; Busse et al., 2012) as well as in animal models of schizophrenia with varying results depending on brain region and age investigated (Juckel et al., 2011; Garay et al., 2013; van den Eynde et al., 2014).

One way in which activated microglia contribute to pathology is through the production of pro-inflammatory cytokines. An imbalance in cytokine levels may trigger aberrant neurodevelopment in the fetus and lead to neuropathology and psychopathology in the adult offspring. Infection-induced increase of pro-inflammatory maternal cytokines may be one of the key events leading to enhanced risk for neuropsychiatric disorders in the offspring (Gilmore and Jarskog, 1997). Human studies revealed that increased maternal serum levels of the pro-inflammatory cytokine Tumor Necrosis Factor-α (TNF-α) and the chemokine Interleukin-8 (IL-8) during pregnancy are directly associated with a higher risk for schizophrenia

* Corresponding author. Address: Max-Delbrück-Center of Molecular Medicine, Cellular Neuroscience, Robert-Rössle-Str. 10, 13125 Berlin, Germany. Tel.: +49 30 94063260; fax: +49 30 94063819.
E-mail address: susanne.wolf@mdc-berlin.de (S.A. Wolf).
[1] DM & ADI/CW & SAW contributed equally as first or last authors respectively.

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in the progeny (Brown et al., 2004; Buka et al., 2001). In line with this finding, *Mednick* and colleagues reported that fetuses gestating during a viral epidemic are at elevated risk for developing schizophrenia (Mednick et al., 1988). Subsequent prospective studies have shown that maternal infections of various types increase the risk for schizophrenia in the offspring three- to sevenfold (for review see Brown and Derkits, 2010). Rodent studies have confirmed that a maternal immune response is sufficient to induce psychopathology in later life (Biscaro et al., 2012; Ozawa et al., 2006; Meyer et al., 2005; Zuckerman and Weiner, 2005; Shi et al., 2003; Abazyan et al., 2010; Zuckerman et al., 2003; Frick et al., 2013). Injection of pregnant rodents with the viral mimic polyinosinic–polycytidilic acid (Poly I:C) leads to a wide spectrum of schizophrenia-relevant behavioral deficits, such as pre-pulse inhibition of the acoustic startle response (PPI) (Gal et al., 2009; Klein et al., 2013; Kumari et al., 2008; Smith et al., 2007; Nyffeler et al., 2006; Schwarzkopf et al., 1992; for review see Yamada, 2000). Behavioral deficits are associated with schizophrenia-relevant neuropathological deficits including abnormalities in dopaminergic and glutamatergic neurotransmission (Winter et al., 2008; for review see Kirkpatrick, 2013), histopathological (Biscaro et al., 2012; Kühn et al., 2012) and structural changes (Piontkewitz et al., 2011a, 2012). The relevance of maternal Poly I:C -induced deficits to schizophrenia is further supported by the responsiveness of adult behavioral deficits to neuroleptic treatment (Piontkewitz et al., 2011b). Finally, prenatal Poly I:C -induced behavioral abnormalities exhibit the maturation delay of schizophrenia (Meyer, 2013, 2014; Feldon and Weiner, 2009), enabling the elucidation of progressive mechanisms possibly underlying behavioral manifestations as well as preventive interventions. Maternal immune stimulation is thus an excellent model to study pathophysiological and therapeutic aspects relevant to schizophrenia (Meyer and Feldon, 2012; Lipina et al., 2013; for reviews see Meyer, 2013, 2014).

Schizophrenia has the most robust clinical evidence for a disease-related reduction in grey and white matter including smaller hippocampal volume assessed in chronic schizophrenic patients (Wexler et al., 2009) and animal models (Meyer, 2013,2014; Piontkewitz et al., 2012; Lipska, 2004). Hippocampal involvement is likely to be associated with neuropsychological impairments of schizophrenia (Harrison, 2004) as well as with its psychotic symptoms (Ewing and Winter, 2013; Floresco and Jentsch, 2011).

Hippocampal structural pathology in schizophrenia might be due to aberrant neurodevelopment and abnormal neural plasticity. One particular example of cell-based brain plasticity is the generation of new neurons in the hippocampus throughout life (Altman and Das, 1965; Eriksson et al., 1998). Neurogenesis has been linked with hippocampal-dependent function (for reviews see Deng et al., 2010; Bruel-Jungerman et al., 2007). Recently, microglial activity has been shown to be important for the homeostasis of neurogenesis, predominantly through the phagocytosis of apoptotic neuronal progenitor cells (Sierra et al., 2010) and balancing apoptotic and proliferative events via TNF-α signaling (Chen and Palmer, 2013). Baseline microglial activity and cytokine levels in the hippocampus are needed to maintain baseline neurogenesis while an immune response accompanied by an increase in pro-inflammatory cytokines is thought to be detrimental for neurogenesis. Consequently, anti-inflammatory drugs have been shown to ameliorate the decrease of neurogenesis caused by pro-inflammatory cytokines (for review see Kohman and Rhodes, 2013). In schizophrenic patients *Miyaoka* and colleagues demonstrated significant and robust clinical improvements using the tetracycline minocycline – a potent inhibitor of microglial activation (Miyaoka et al., 2008; Seki et al., 2013). Minocycline has been used successfully in some clinical trials since as an adjunctive therapy to antipsychotics for schizophrenia (for review see Dean et al., 2012). How minocycline affects microglia function *in vivo* and neurogenesis is still not fully understood.

We here evaluated the effects of minocycline treatment on neurogenesis in parallel to microglia density, activation and cytokine production in the hippocampus compared to other brain regions in the Poly I:C rat model of schizophrenia. We correlated these data with the effects of minocycline treatment on sensorimotor gating deficits – a behavioral phenotype relevant to schizophrenia.

2. Materials and methods

2.1. Animals

All experimental protocols conformed to the guidelines of the European Communities Council Directive (86/609/EEC) for care of laboratory animals and were approved by the local ethic committee (Landesdirektion Dresden). Wistar Rats (Harlan laboratories) were housed in a temperature and humidity controlled vivarium with a 12-h light–dark cycle (lights on: 6 a.m. to 6 p.m.). They had access to food and water *ad libitum*.

2.2. Poly I:C injections

Rats were mated at about an age of three months and the first day after copulation was defined as day one of pregnancy. On gestation day 15, pregnant rats received a single i.v. injection to the tail vein of either Poly I:C (4.0 mg/kg, SIGMA, Germany) dissolved in 200 µl 0.9% NaCl, or vehicle (Klein et al., 2013). On postnatal day (PND) 21, pups were weaned and housed by sex and litter and left undisturbed until behavioral phenotyping at PND 90–98. Prior to the experiments rats were handled for about ten minutes daily for three days. Handling comprised habituation to the investigator and the startle chamber. Each experimental group consisted of male subjects derived from multiple independent litters, with no more than three rats from the same litter. The following experimental groups were included into the study: NaCl H_2O – animals derived from mothers injected with 0.9% NaCl during pregnancy and during treatment period supplied with drinking water. NaCl mino – animals derived from mothers injected with 0.9% NaCl during pregnancy and treated with minocycline added to drinking water. Poly I:C H_2O –animals derived from mother injected with Poly I:C during pregnancy and during treatment period supplied with drinking water. Poly I:C mino – animals derived from mothers injected with Poly I:C during pregnancy and treated with minocycline added to drinking water.

2.3. Minocycline treatment

Minocycline was added to the drinking water from PND 60 until 128, when animals were sacrificed. With an average of 5 ml water intake per 250 g rat per day, the animals received an average daily dosage of 3 mg/kg over the course of approximately 70 days. The daily dosage was adapted from treatments in schizophrenic patients (Levkovitz et al., 2010). Water bottles were changed every second day to ensure minocycline stability.

2.4. BrdU injection

Five animals of each group received i.p. injections of 10 mg/ml BrdU at 50 mg/kg body weight for three consecutive days starting at PND 98 to label proliferating cells to be subsequently analyzed for levels of neurogenesis at PND 128.

2.5. Immunohistochemistry

The standard procedure for evaluation of cell proliferation and neurogenesis including analysis had been described elsewhere