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Hippocampal structure and function are maintained despite severe innate peripheral inflammation

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ABSTRACT

Chronic peripheral inflammation mediated by cytokines such as TNFα, IL-1β, and IL-6 is associated with psychiatric disorders like depression and anxiety. However, it remains elusive which distinct type of peripheral inflammation triggers neuroinflammation and affects hippocampal plasticity resulting in depressive-like behavior. We hypothesized that chronic peripheral inflammation in the human TNF-α transgenic (TNFtg) mouse model of rheumatoid arthritis spreads into the central nervous system and induces depressive state manifested in specific behavioral pattern and impaired adult hippocampal neurogenesis. TNFtg mice showed severe erosive arthritis with increased IL-1β and IL-6 expression in tarsal joints with highly elevated human TNF-α levels in the serum. Intriguingly, IL-1β and IL-6 mRNA levels were not altered in the hippocampus of TNFtg mice. In contrast to the pronounced monocytosis in joints and spleen of TNFtg mice, signs of hippocampal microgliosis or astrocytosis were lacking. Furthermore, locomotion was impaired, but there was no locomotion-independent depressive behavior in TNFtg mice. Proliferation and maturation of hippocampal neural precursor cells as well as survival of newly generated neurons were preserved in the dentate gyrus of TNFtg mice despite reduced motor activity and peripheral inflammatory signature. We conclude that peripheral inflammation in TNFtg mice is mediated by chronic activation of the innate immune system. However, severe peripheral inflammation, though impairing locomotor activity, does not elicit depressive-like behavior. These structural and functional findings indicate the maintenance of hippocampal immunity, cellular plasticity, and behavior despite peripheral innate inflammation.

1. Introduction

A growing number of studies show that chronic inflammatory diseases, *e.g.* rheumatoid arthritis (RA), are frequently associated with neuropsychiatric comorbidities, in particular depression, anxiety, and cognitive dysfunction (Covic et al., 2012; Shin et al., 2012; Matcham et al., 2013; Rathbun et al., 2013). Mediators of an immune response like cytokines TNF-α, IL-1β, IL-2, and IL-6 were reported to trigger changes in behavior, such as sickness behavior, low mood, fatigue, anxiety, anhedonia, and cognitive disorders (Pollak and Yirmiya, 2002; Capuron and Miller, 2004; Dantzer et al., 2008; Pertsov et al., 2009; Karrenbauer et al., 2011). In addition, both TNF-α and its receptor levels are significantly elevated in the peripheral blood and cerebral spinal fluid of depressed patients (Himmerich et al., 2008; Dowlati et al., 2010), and improvement of depression and antidepressant treatment correlates with a decrease in serum level of TNF-α both in animals and human (Lanquillon et al., 2000; Brustolim et al., 2006; Kumar et al., 2011). A pivotal mechanism for compromised central nervous system (CNS) functioning by peripheral inflammation is the induction of neuroinflammation (Dantzer et al., 2008). Suggested mechanisms for the propagation of peripheral inflammation into the CNS include the stimulation of vagal nerve afferents (Bluthe et al., 1994), the transport of cytokines across the blood brain barrier (BBB) (Banks, 2006), and altered NO metabolism leading to BBB breakdown (Najjar et al., 2013). However, it remains elusive which distinct type of peripheral inflammation induces depressive state, triggers neuroinflammation, and potentially affects hippocampal structures and functions. One potential explanation

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for these symptoms is altered adult hippocampal neurogenesis, underlining the need to elucidate how and under which conditions peripheral inflammation is able to influence this process. Indeed, decreased adult hippocampal neurogenesis has been associated with depression, anxiety, and cognitive impairment (Jacobs et al., 2000). Intriguingly, adult hippocampal neurogenesis is a highly sensitive neurobiological process. A broad variety of extrinsic and intrinsic stimuli were shown to influence adult hippocampal neurogenesis, thereby potentially modulating hippocampal function. The generation of new neurons in the adult hippocampus declines with age (Spalding et al., 2013) and is negatively regulated by stress (Jacobs et al., 2000), neuropathic pain (Mutso et al., 2012), and local CNS inflammation induced by irradiation (Monje et al., 2003) or cortical LPS injection (Ekdahl et al., 2003). This repressive effect of neuroinflammation on cellular hippocampal plasticity is mediated by proinflammatory cytokines like TNF-α, which impair the survival of hippocampal neural progenitor cells (NPCs) *in vivo* (Yang et al., 2002) and negatively regulate hippocampal NPC proliferation *in vitro* (Ben-Hur et al., 2003).

To investigate the relationship between peripheral inflammation and structural as well as functional hippocampal changes we used the Tg197 strain of human TNF-α transgenic (TNFtg) mice, a widely used model for rheumatoid arthritis (Keffer et al., 1991).

Our results indicate that TNFtg mice display severe innate peripheral monocytic inflammation. More importantly, TNFtg mice lack signs of hippocampal neuroinflammation and do not reveal any behavioral or cellular (estimated by hippocampal neurogenesis) signs of a depressive state.

2. Materials and methods

2.1. Animals

The tg 197 strain of TNFtg mice was generated by integration of the human TNF-α (hTNF) gene into the mouse genome. Substitution of the regulatory 3′-UTR by the human β-globin gene induces constitutive expression of the transgene by mesenchymal cells, predominantly by the monocytic lineage (Keffer et al., 1991). Besides high expression in the joints of TNFtg mice, expression of hTNF mRNA was detected in brain, thymus, spleen, kidney, and lung of the tg 197 strain (Keffer et al., 1991). TNFtg mice mimic important aspects of RA including chronic progressive polyarthritis and locomotor impairment. Joint pathology of TNFtg mice starts with generalized joint swelling at the age of 4 weeks. The life expectancy of TNFtg mice is about 16 weeks (Keffer et al., 1991). Notably, there is no difference in the development of arthritis between male and female TNFtg mice. Inflammatory pain in TNFtg mice is evident from enhanced nociceptive behavior after exposure to thermal and mechanic stimuli (Hess et al., 2011).

To yield homogenous groups of wt and TNFtg mice for most experiments, female wt mice (C57BL/6) were bred with male heterozygous TNFtg mice (C57BL/6, strain Tg197) (Keffer et al., 1991). The female wt and heterozygous TNFtg offspring served as experimental animals. In case of the performed behavior tests, both male and female wt and TNFtg mice were chosen to receive a sufficient number of experimental animals per group. All mice were kept in a light-dark cycle of 12 h and had free access to food and water. Mice were anaesthetized and transcardially perfused with 0.9% NaCl prior to tissue harvesting.

All experiments were carried out in accordance with the National Institutes of Health guidelines for the humane treatment of animals and the European Communities Council Directive (86/609/EEC) and approved by the local governmental commission for animal health.

2.2. TNF serum levels and leukocyte count

To determine hTNF-α serum levels, blood of 6- and 12-week-old female wt and TNFtg mice was drawn ($n = 5$ per group and age). Serum was processed as described previously (Polzer et al., 2011) and hTNF-α levels were measured with the multiplex bead array technology (Bender MedSystems, Vienna, Austria).

To determine the distribution of leukocyte subtypes, blood smears were performed of blood was drawn from female wt ($n = 3$) and TNFtg ($n = 5$) mice between 8 and 12 weeks of age. Blood smears were generated and proportional distributions of leukocyte subtypes were counted.

2.3. Experiment I – Morphological correlates of arthritis

12-week-old female mice ($n = 7$ per group) were subjected to micro-CT analysis of the hindpaw. Micro-CT imaging and 3D-reconstruction were conducted as previously described (Chen et al., 2013). Afterwards, both hind paws were dissected and fixed overnight in 4% PFA to stain with hematoxylin and eosin (H&E) and to perform immunohistochemical F4/80 staining for monocytes/macrophages.

2.4. Experiment II – Peripheral monocytes in joints and spleen

Hindpaws and spleens of 12-week-old female animals ($n = 10$ per group) were dissected for flow cytometric analysis of peripheral monocytes/macrophages.

2.5. Experiment III – Inflammation in joints and brain

Brains of 12-week-old female mice ($n = 3$ per group) were removed. Right hemispheres were stored at −80 °C for protein analysis. Left hemispheres were assigned for RNA analysis. Cortex and hippocampus were dissected and stored at −80 °C. Hindpaws were dissected to analyze RNA expression of cytokines in tarsal joints.

2.6. Behavioral testing

Animals were submitted to a battery of behavioral tests in the following order: open field, light-dark box, elevated plus maze, novelty suppressed feeding, forced swim, and sucrose preference tests. Complete behavioral testing lasted for about 3 weeks. We chose to start behavioral testing with 7-week-old mice, as a robust phenotype of TNFtg mice including hTNF overexpression was already present at that age. Moreover, prescreening of motor function confirmed that mobility of TNFtg mice was not apparently impaired until the end of the test battery. All tests were performed on separate days between 09:00 and 14:00 h. Group sizes were identical for all tests (wt: $n = 11$; TNFtg: $n = 9$). Mice were tested in a pseudorandom order and were moved to the behavioral suite adjacent to the housing room immediately before testing. Each test apparatus was cleaned with 5% ethanol between subjects to avoid any olfactory cues influencing behaviors. Mice were returned to their home cages at the end of each test and allowed to recover for at least 2 days before further testing. Behaviors for all tests were recorded on videotape for subsequent scoring.

2.7. Experiment V – Neurogenesis and glial neuroinflammatory response

Eight-week-old female animals ($n = 4$ per group) received doses of 50 mg/kg of the nucleoside analogue 5-bromo-2′-deoxyuridine (BrdU) (Sigma–Aldrich, St. Louis, MO, USA) by intraperitoneal injections on five consecutive days. Four weeks after the last

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