Brain, Behavior, and Immunity 31 (2013) 134–142

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Indoleamine 2,3-dioxygenase inhibition attenuates lipopolysaccharide induced persistent microglial activation and depressive-like complications in fractalkine receptor (CX_3CR1)-deficient mice

Angela W. Corona [a], Diana M. Norden [a], John P. Skendelas [a], Yan Huang [a], Jason C. O'Connor [b], Marcus Lawson [c], Robert Dantzer [c], Keith W. Kelley [c], Jonathan P. Godbout [a,d,e,*]

[a] Department of Neuroscience, The Ohio State University, 333 W. 10th Ave., Columbus, OH 43210, USA
[b] Department of Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229, USA
[c] Department of Animal Science, University of Illinois, 1201 W. Gregory Drive, 250B Edward R. Madigan Laboratory, Urbana, IL 61820, USA
[d] Institute for Behavioral Medicine Research, The Ohio State University, 460 Medical Center Dr., Columbus, OH 43210, USA
[e] Center for Brain and Spinal Cord Repair, The Ohio State University, 460 W. 12th Ave., Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Available online 19 August 2012

Keywords:
Microglia
Depression
Indoleamine 2,3-dioxygenase
Kynurenine
Fractalkine receptor
1-Methyl-tryptophan

ABSTRACT

An impaired ability to regulate the activation of microglia by fractalkine (CX_3CL1) leads to persistent neuroinflammation and behavioral alterations following lipopolysaccharide (LPS) challenge. While these responses are usually transient, LPS injection caused prolonged depressive-like behavior in fractalkine receptor deficient mice ($CX_3CR1^{-/-}$) that was associated with exaggerated microglial activation and induction of the tryptophan (TRP) degrading enzyme indoleamine 2,3-dioxygenase (IDO). IDO activation and subsequent generation of neuroactive kynurenine metabolites may have a pivotal role in the development of depression. Therefore, the purpose of this study was to determine the extent to which LPS-induced depressive-like behavior in $CX_3CR1^{-/-}$ mice was dependent on IDO activation. $CX_3CR1^{-/-}$ mice were implanted prior to LPS challenge with a slow release pellet of 1-methyl-tryptophan (1-MT), a competitive inhibitor of IDO. Here we show that the depressive-like behavior evident in $CX_3CR1^{-/-}$ mice 72 h after LPS injection was abrogated by inhibition of IDO. LPS also decreased body weight and locomotor activity in $CX_3CR1^{-/-}$ mice, but these effects were independent of 1-MT. Consistent with the increased metabolism of TRP by IDO, the ratio of 3-hydroxykynurenine (3-HK) to TRP was increased in the brain 72 h after LPS. Increased serotonin (5-HT) turnover was also evident in the brain. The LPS-associated increases in both 3-HK:TRP and 5-HIAA:5-HT ratios were prevented by the inhibition of IDO. Last, IDO blockade attenuated microglial activation in the prefrontal cortex and hippocampus 72 h after LPS. Collectively these data indicate that LPS-induced IDO activation contributes to persistent microglial activation and depressive-like behavior in $CX_3CR1^{-/-}$ mice.

Published by Elsevier Inc.

1. Introduction

Microglia are resident innate immune cells of the CNS that are important in immune surveillance (Davalos et al., 2005; Nimmerjahn et al., 2005) and help to interpret and propagate inflammatory signals in the brain. For example, activated microglia produce pro-inflammatory cytokines and secondary messengers that promote physiological and behavioral responses including sickness behavior (Dantzer et al., 2008). The cytokine mediated sickness response is normally adaptive, but amplified or prolonged microglial activation is associated with behavioral and cognitive complications (Corona et al., 2011). For this reason it is critical that the activation of microglia is tightly regulated. There are several key regulatory systems within the brain that modulate microglia activation including fractalkine (CX_3CL1) production by neurons.

Complementary expression of CX_3CL1 on neurons and fractalkine receptor (CX_3CR1) on microglia (Hughes et al., 2002; Maciejewski-Lenoir et al., 1999; Nishiyori et al., 1998; Pan et al., 1997) establishes a unique communication system whereby neurons constitutively express CX_3CL1 to regulate the activation of microglia (Cardona et al., 2006). In support of this notion, CX_3CR1 deletion ($CX_3CR1^{-/-}$) increased the inflammatory profile of the brain and was associated with decreased neurogenesis, reduced long term potentiation, and deficits in motor and spatial learning (Bachstetter et al., 2011; Rogers et al., 2012). Moreover, several rodent models indicate that CX_3CL1–CX_3CR1 interactions are

* Corresponding author at: Institute for Behavioral Medicine Research, The Ohio State University, 460 Medical Center Dr., Columbus, OH 43210, USA. Tel.: +1 614 293 3456; fax: +1 614 366 2097.
E-mail address: godbout.2@osu.edu (J.P. Godbout).

0889-1591/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbi.2012.08.008

impaired with normal aging (Bachstetter et al., 2011; Lyons et al., 2009; Wynne et al., 2010).

The interactions between fractalkine (CX_3CL1) and the corresponding fractalkine receptor (CX_3CR1) are particularly important when microglia become activated by an inflammatory challenge (Cardona et al., 2006; Mizuno et al., 2003; Zujovic et al., 2001; Zujovic and Taupin, 2003). In aged mice, peripheral injection of LPS caused protracted down-regulation of CX_3CR1 expression on microglia that corresponded with amplified IL-1β mRNA levels compared to adult mice (Wynne et al., 2010). Similar to previous studies in older mice (Godbout et al., 2005, 2008), $CX_3CR1^{-/-}$ mice had prolonged sickness behavior (24 h) and depressive complications (72 h) following a single i.p. injection of LPS (Corona et al., 2010). Furthermore, the LPS-induced prolonged depressive-like behavior and microglial activation in $CX_3CR1^{-/-}$ mice was associated with an amplified induction of indoleamine 2,3-dioxygenase (IDO) and kynurenine monooxygenase (KMO) mRNA (Corona et al., 2010). These two enzymes are involved in the degradation of tryptophan into several neuroactive metabolites that influence the inflammatory state of the CNS and affect behavioral responses (Haroon et al., 2012).

IDO is activated by inflammatory cytokines (i.e., IFNα, IFNγ, IL-1, TNFα) and provides the initial enzymatic activity in the degradation of tryptophan into several metabolites that have the potential to disrupt several key neurotransmitter systems in the CNS. In the brain, active IDO in microglia converts tryptophan (TRP) to kynurenine (KYN) (Guillemin et al., 2005) and then KYN is further processed by KMO to produce the neuroactive metabolites, 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN). Because the rate liming step in the synthesis of serotonin (5-HT) involves the catabolism of TRP by tryptophan hydroxylase, the reduction in TRP may affect the concentrations of 5-HT. In addition, increased levels of KYN-related metabolites (3-HK and QUIN) are associated with increased lipid peroxidation, agonism of NMDA glutamate receptors and disruption of dopamine signaling (Dantzer et al., 2011; Haroon et al., 2012; Muller and Schwarz, 2007). Because these neurotransmitters are affected, activation of the IDO pathway has been linked to depressive-like complications in rodents (O'Connor et al., 2009a, 2009b, 2009c) and humans (Capuron et al., 2011; Raison et al., 2010; Steiner et al., 2011). For example, Bacille Calmette-Guerin (BCG) infection or LPS injection in CD-1 mice caused depressive-like behavior that was blocked by 1-methyl-tryptophan (1-MT), a tryptophan analog and competitive inhibitor of IDO (O'Connor et al., 2009b, 2009c). In humans, interferon (IFN)-α therapy for hepatitis C patients is associated with depressive complications (Haroon et al., 2012). IFN-α increases the KYN concentration in the blood and the cerebrospinal fluid (CSF) (Capuron et al., 2011; Raison et al., 2010) and the increased levels of KYN in the CSF of depressed patients positively correlated with depressive symptoms (Raison et al., 2010).

Based on these data, we hypothesized that an impaired ability to regulate microglia by CX_3CR1 causes prolonged IDO activation in response to LPS and the subsequent downstream processing of KYN, leading to the development and persistence of depressive-like behavior. Here we show that inhibition of IDO with 1-MT attenuated microglial activation in the pre-frontal cortex and hippocampus and blocked depressive-like behavior induced in $CX_3CR1^{-/-}$ mice by LPS injection.

2. Materials and methods

2.1. Animals

Adult (3–6 mo) C57BL/6 fractalkine receptor deficient mice ($CX_3CR1^{-/-}$) from our in-house specific pathogen free colony were used (Corona et al., 2010). Mice were housed in polypropylene cages and maintained at 25 °C under a reverse-phase, 12 h light/12 h dark cycle with *ad libitum* access to water and rodent chow. Mice were individually housed 2-weeks prior to implantation with placebo or 1-MT pellets and remained individually housed for the duration of the experimental procedures. All procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.2. Experimental design

In these experiments, adult $CX_3CR1^{-/-}$ mice were implanted subcutaneously (s.c.) with a slow release pellet filled with either placebo or 1-methyl-DL-tryptophan (1-MT; Sigma) as previously described (O'Connor et al., 2009b, 2009c). Pellets were purchased pre-filled from Innovative Research of America (Sarasota, FL) and were designed to deliver 5 mg/day of drug or placebo for 7 days (O'Connor et al., 2009b, 2009c). After 3 days of 1-MT or placebo pretreatment, mice were injected intraperitoneally (i.p.) with saline or LPS (0.5 mg/kg; serotype 0127:B8, Sigma, St. Louis, MO). This LPS dosage was selected because it elicits a pro-inflammatory cytokine response in the brain resulting in a transient sickness response in wild type and $CX_3CR1^{+/-}$ mice (Corona et al., 2010). Locomotor activity, body weight, and food intake were determined 0, 24, 48, and 72 h after injections ($n = 8$). At 72 h after injections, depressive-like behavior was determined using the tail suspension test ($n = 8$–10). Within 2 h of the completion of behavioral testing, a subset of mice were sacrificed and the brain was collected to determine 1-MT, TRP, KYN, 3-HK, 5-HT and 5-hydroxyindoleacetic acid (HIAA) levels ($n = 4$–8). A second cohort of mice were transcardially perfused with sterile phosphate-buffered saline (PBS, pH 7.4 w/EDTA) followed by fixation with 4% formaldehyde. Brains were removed, processed, and labeled with an anti-Iba-1 antibody ($n = 4$).

2.3. Behavior

Locomotor activity and depressive-like behavior were determined as previously described (Godbout et al., 2005, 2008; O'Connor et al., 2009c). In brief, mice were acclimated to routine handling for 5 days before experimentation. Behavioral tests were conducted during the dark phase (between 1200 and 2400) of the photoperiod under infrared lighting in the same room in which the mice were normally housed. Behavior was scored by a trained observer who was blind to the experimental treatments.

For locomotor activity, mice were maintained in their home cage with a floor area of 26 × 20 cm, and activity was video recorded for 3 min. On the video records, cages were divided into sixidentical virtual rectangles and the number of line crossings was determined.

For depressive-like behavior, the tail suspension test (TST) was used. Mice were suspended by their tail in a 32 × 33 × 33 cm box and the duration of immobility was determined over a 10 min period (Corona et al., 2010; Godbout et al., 2008; O'Connor et al., 2009c). Time spent immobile was determined by a trained observer who was blind to the experimental treatments. The first 2 min were omitted to allow the mice to acclimate to the testing procedures. Results are expressed as the total immobility for the last 8 min of the test.

2.4. Neurochemistry

Plasma and brain levels of KYN, TRP, and 1-MT were determined as previously described (O'Connor et al., 2009c). For the plasma