Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Altered microglia morphology and higher resilience to stress-induced depression-like behavior in CX3CR1-deficient mice

Sabine Hellwig [a,*], Simone Brioschi [a], Sandra Dieni [a], Lars Frings [b,c], Annette Masuch [a], Thomas Blank [d], Knut Biber [a,e,*]

[a] Department of Psychiatry and Psychotherapy, University Hospital Freiburg, Freiburg, Germany
[b] Centre of Geriatrics and Gerontology, University Hospital Freiburg, Freiburg, Germany
[c] Department of Nuclear Medicine, University Hospital Freiburg, Freiburg, Germany
[d] Department of Neuropathology, University Hospital Freiburg, Freiburg, Germany
[e] Department for Neuroscience, University Medical Center Groningen, University of Groningen, Netherlands

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 6 November 2015
Accepted 9 November 2015
Available online xxxx

Keywords:
Depression
Fractalkine
CX3CR1
CX3CL1
Stress
Microglia

ABSTRACT

Microglia are suggested to be involved in several neuropsychiatric diseases. Indeed changes in microglia morphology have been reported in different mouse models of depression. A crucial regulatory system for microglia function is the well-defined CX3C axis. Thus, we aimed to clarify the role of microglia and CX3CR1 in depressive behavior by subjecting CX3CR1-deficient mice to a particular chronic despair model (CDM) paradigm known to exhibit face validity to major depressive disorder. In wild-type mice we observed the development of chronic depressive-like behavior after 5 days of repetitive swim stress. 3D-reconstructions of Iba-1-labeled microglia in the dentate molecular layer revealed that behavioral effects were associated with changes in microglia morphology towards a state of hyper-ramification. Chronic treatment with the anti-depressant venlafaxine ameliorated depression-like behavior and restored microglia morphology. In contrast, CX3CR1 deficient mice showed a clear resistance to either (i) stress-induced depressive-like behavior, (ii) changes in microglia morphology and (iii) antidepressant treatment. Our data point towards a role of hyper-ramified microglia in the etiology of chronic depression. The lack of effects in CX3CR1 deficient mice suggests that microglia hyper-ramification is controlled by neuron-microglia signaling via the CX3C axis. However, it remains to be elucidated how hyper-ramified microglia contribute to depressive-like behavior.

1. Introduction

Major depression is the most common form of mental illness affecting modern society. A multifactorial etiology of depression has been postulated, whereby psychosocial stress, genetic predisposition, personality and somatic co-factors are suggested to contribute to the underlying neuropathological substrate. Furthermore, recent studies have provided evidence for disseminated changes occurring in transmitter systems, endocrinological circuits and inflammatory cascades (Dantzer et al., 2008; Krishnan and Nestler, 2008; Kupfer et al., 2012).

The hippocampus has been identified as a key player in the neurobiological regulation of depressive behavior (Small et al., 2011). Moreover, reduced hippocampal volume has previously been found in a subset of depressed patients (Campbell et al., 2004; Videbech and Ravnkilde, 2004). These findings have formed the basis of the well-recognized neuroplasticity hypothesis in depression, which involves for example decrements in neurotrophic factors that regulate adult synaptic plasticity (Duman and Monteggia, 2006; Monteggia et al., 2004).

The pathophysiological contribution of microglia to depressive disorders is becoming a topic of growing interest, for example, changes in microglial morphology have been linked to depression-like behavior in mice and rats (see for review: (Walker et al., 2013; Yirmiya et al., 2015). Furthermore, it was proposed that the inhibition of microglia function either by minocycline treatment or transgenic overexpression of IL1-RA in the brain leads to changes in depression-like behavior in rodents (Hinwood et al., 2013; Kreisel et al., 2013). Since microglia are a major source of pro-inflammatory mediators in the CNS, these cells are central to the theory that depression is a manifestation of a

* Corresponding authors at: Department of Psychiatry and Psychotherapy, University Hospital Freiburg, Hauptstr. 5, 79104 Freiburg, Germany.
E-mail addresses: sabine.hellwig@uniklinik-freiburg.de (S. Hellwig), knut.biber@uniklinik-freiburg.de (K. Biber).

http://dx.doi.org/10.1016/j.bbi.2015.11.008
0889-1591/

dysfunctional immune system (Leonard, 2010). Intriguingly, microglia have been suggested to play a role in the regulation of synaptic plasticity (Paolicelli et al., 2011).

A crucial regulatory system for microglia function is the well-defined CX3C axis (for review see: (Wolf et al., 2013)). While microglia are characterized by prominent expression of the chemokine receptor CX3CR1, cerebral synthesis and secretion of its ligand CX3CL1/fractalkine is mostly restricted to neurons. A deficiency in either the CX3C receptor or ligand dramatically changes microglia reactivity in various brain disease models, most of which result in deleterious effects. Under physiological conditions it was found that $CX3CR1^{GFP/GFP}$ mice (CX3CR1-deficient) exhibit alterations in developmental synaptic pruning (Paolicelli et al., 2011). Furthermore, previous research indicates that CX3CR1 deficiency promoted depression-like behavior in response to peripheral inflammation, which was, in turn, associated with protracted morphological microglial activation (Corona et al., 2010). To date, few studies have evaluated the behavioral outcome of conditions that resemble human depressogenic risk factors (e.g. chronic stress) in the CX3CR1 deficient mice.

Against this background, the aims of the present study were:

1. To investigate depression-like behavior and microglia responses following chronic stress in $CX3CR1^{GFP/GFP}$ mice *versus* wild-type animals.
2. To compare the antidepressant treatment response to venlafaxine in $CX3CR1^{GFP/GFP}$ mice and wild-type animals.

2. Methods

2.1. Animals & Ethics

Experiments were performed in agreement with the German law on the use of laboratory animals and in accordance with the institutional guidelines of the University of Freiburg. The use of animals was approved both by the Regional Council of Freiburg (Regierungspräsidium) and the local animal care authorities. CX3CR1 mutants (Jung et al., 2000) were maintained on a C57BL/6 J background. The day of birth was considered as postnatal day (P) 0. The colony was inbred for more than ten generations. Appropriate handling was undertaken in accordance with guidelines from our Regional Council and the local animal care authorities. Mice were bred under specific pathogen-free conditions in a temperature- and humidity-controlled vivarium with a 12 h light-dark cycle. Food and water were available ad libitum. In all experiments wild-type and mutant animals were littermates. Both male and female animals were used at the age of 2–3 months.

2.2. Immunohistochemistry

2.2.1. Staining procedure

Mice were anesthetized with ketamine hydrochloride (Ketamin, CP Pharma, Burgdorf, Germany; 100 mg/kg body weight) and xylazine (Rompun, Bayer Vital GmbH, Leverkusen, Germany; 20 mg/kg body weight) and transcardially perfused with ice-cold PBS within 1 h of the last behavioral experiment. Dissected brains were fixed in 4% PFA and subsequently cryoprotected in 30% sucrose solution. Both steps were performed at 4 °C for 48 h each.

Brains were cut coronally on a cryostat (50 μm) and serial sections of the hippocampus collected. For immunohistochemistry we randomly selected 2 sections per animal from the dorsal hippocampus.

Free-floating sections were pre-incubated for 60 min in blocking solution (10% horse serum (HS) containing 0.5% Triton X-100 in PBS) at room temperature (RT). Subsequently, sections were incubated with the primary antibodies in 10% HS in PBS overnight at 4 °C. After washing (3 × 10 min in PBS) at RT, sections were incubated in secondary fluorochrome-conjugated antibodies in PBS for 2 h at RT. For nuclear counterstain, 1 μg/ ml of 4′, 6-Diamidin-2-phenylindol (DAPI) was applied for 10 min. After rinsing in PBS (3 × 10 min), sections were mounted on Superfrost glass slides and coverslipped with fluorescent mounting medium (DAKO, Glostrup, Denmark).

For detection of activity-regulated cytoskeleton protein (Arc/Arg 3.1), an antigen-recovery protocol using acid treatment was applied as previously described (Jiao et al., 1999; Junghans et al., 2005).

2.2.2. Antibodies

The following primary antibodies were used for immunohistochemistry: rabbit anti-Iba1 (1:1000; 019-19741, Wako Chemicals GmbH, Neuss, Germany), and guinea pig anti-Arc/Arg 3.1 (1:1000; 156005, Synaptic Systems, Göttingen, Germany). Secondary antibodies: Alexa-Fluor series (each 1:1000; A-21424, A-11008, Life Technologies, Darmstadt, Germany), donkey anti-guinea pig IgG Cy5 (1:400; AP193S, Life Technologies).

2.2.3. Imaging, quantification and data analysis of immunohistochemistry

Immuno-stained sections were analyzed by confocal laser scanning using a ZEISS LSM 510 META microscope. High magnification and z-stack images were obtained with an LD LCI Plan-Apochromat 25×/0.8 Imm Korr. DIC objective (Zeiss). Imaging speed was 4 (pixel dwell 12.8 μs) with a resolution of 1024 × 1024 pixels. For 3D-reconstruction of microglia, z-stacks of 30 μm thickness, each with a z-slice interval of 0.8 μm were captured. Acquired images were minimally adjusted for contrast and brightness levels in Adobe Photoshop.

The density of Arc/Arg 3.1-labeled cells was determined in 2 coronal sections of the dorsal hippocampus per animal ($n = 3$ animals per genotype and treatment). Single plane confocal images were used and cell numbers were manually counted in the granule cell layer of the dentate gyrus (DG) bilaterally using the ImageJ multi-points selection tool. The area of interest, (granule cell layer), was encircled using the freehand tool and measured using ImageJ 1.40 analysis software (National Institutes of Health). Cell densities are expressed as the number of cells per area.

Quantification of the density of Iba1+ profiles in the superior and inferior molecular layer (ML) of the DG was performed using ImageJ 1.40 analysis software (n = at least 3 animals per genotype and condition, 2 random sections per animals from dorsal hippocampus; approximately between bregma −2.12 and −2.92 (Mouse Brain Atlases (http://www.mbl.org)). Single plane confocal images were taken from the DG region. All imaging parameters were the same for each image. The images were converted into a binary black and white format using the ImageJ processing tool "Make binary" with an automated threshold ("default" threshold, a variation of the IsoData algorithm also known as iterative intermeans). The region of interest was defined using the freehand tool. An automated count of black pixels (representing Iba1+ signals) was applied and the Iba1 staining was reported as percentage of area. The measurements from the superior and inferior ML were pooled and the mean value reported. Microglial cells in the DG were counted by tracking the number of double positive Iba1 (microglial cytoplasm, red channel)/DAPI (nuclei, blue channel) structures. Automated colocalization was performed using Bitplane Imaris 7. The mean number of cells per mm^2 was derived from 8 confocal images per group.

Three-dimensional (3D)-reconstructions were performed using IMARIS Filament Tracer (www.bitplane.com), as previously described (Vinet et al., 2012). The z-stack was uploaded to the IMARIS-program, rendering a 3D volume. Cells from the inferior

Please cite this article in press as: Hellwig, S., et al. Altered microglia morphology and higher resilience to stress-induced depression-like behavior in CX3CR1-deficient mice. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2015.11.008