



## Cannabinoid receptor 2 deficiency results in reduced neuroinflammation in an Alzheimer's disease mouse model



Anne-Caroline Schmöle<sup>a,b</sup>, Ramona Lundt<sup>a</sup>, Svenja Ternes<sup>a</sup>, Önder Albayram<sup>a</sup>, Thomas Ulas<sup>c</sup>, Joachim L. Schultze<sup>c</sup>, Daniele Bano<sup>b</sup>, Pierluigi Nicotera<sup>b</sup>, Judith Alferink<sup>a,d,e</sup>, Andreas Zimmer<sup>a,\*</sup>

<sup>a</sup> Institute of Molecular Psychiatry, University of Bonn, Bonn, Germany

<sup>b</sup> German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

<sup>c</sup> Life and Medical Sciences (LIMES) Institute, Genomics and Immunoregulation, University of Bonn, Germany

<sup>d</sup> Department of Psychiatry, University Münster, Münster, Germany

<sup>e</sup> Cluster of Excellence EXC 1003, Cells in Motion, Münster, Germany

### ARTICLE INFO

#### Article history:

Received 11 April 2014

Received in revised form

1 September 2014

Accepted 17 September 2014

Available online 28 September 2014

#### Keywords:

Alzheimer's disease

CB2

CCL2

Endocannabinoid system

Microglia

Neuroinflammation

### ABSTRACT

Several studies have indicated that the cannabinoid receptor 2 (CB2) plays an important role in neuroinflammation associated with Alzheimer's disease (AD) progression. The present study examined the role of CB2 in microglia activation in vitro as well as characterizing the neuroinflammatory process in a transgenic mouse model of AD (APP/PS1 mice). We demonstrate that microglia harvested from CB2<sup>-/-</sup> mice were less responsive to pro-inflammatory stimuli than CB2<sup>+/+</sup> microglia, based on the cell surface expression of ICAM and CD40 and the release of chemokines and cytokines CCL2, IL-6, and TNF $\alpha$ . Transgenic APP/PS1 mice lacking CB2 showed reduced percentages of microglia and infiltrating macrophages. Furthermore, they showed lowered expression levels of pro-inflammatory chemokines and cytokines in the brain, as well as diminished concentrations of soluble A $\beta$  40/42. The reduction in neuroinflammation did not affect spatial learning and memory in APP/PS1\*CB2<sup>-/-</sup> mice. These data suggest a role for the CB2 in Alzheimer's disease-associated neuroinflammation, independent of influencing A $\beta$ -mediated pathology and cognitive impairment.

© 2015 Elsevier Inc. All rights reserved.

### 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and represents the most common type of dementia among the elderly population. Neuropathological hallmarks characteristic of AD include amyloid- $\beta$  (A $\beta$ ) plaques and neurofibrillary tangles, accompanied by neuroinflammation characterized by astrocytosis and microgliosis. Microglia attracted to A $\beta$  deposits represent the primary cellular component associated with AD neuroinflammation. In response to immune-stimulatory signals, they change from a resting to an activated state. Activated microglia express at least 2 phenotypes depending on their environmental stimulation: an M(IFN $\gamma$ ), formerly known as M1, phenotype associated with the production of proinflammatory mediators and an M(IL-4), formerly known as M2, alternatively activated phenotype characterized by anti-inflammatory qualities (Michelucci et al.,

2009; Murray et al., 2014). Several studies have shown that A $\beta$ -mediated activation of microglia induces the production of various chemokines and cytokines, neurotoxic secretory products, free radical species, and NO intermediates (Heneka, et al., 2010). These proinflammatory mediators cause neuronal dysfunction and cell death, suggesting that activation of microglia plays a prominent role in neuroinflammation in the context of AD. In addition to activation of the pro-inflammatory cascade, microglia cells have also been shown to phagocytize A $\beta$  plaques, thereby reducing the number of protein aggregates in an AD brain (Bolmont et al., 2008).

The endocannabinoid system (ECS) is a retrograde messenger system consisting of lipid signaling molecules that bind to at least 2 G-protein-coupled receptors. Cannabinoid receptor 1 (CB1) is mainly expressed in the central nervous system (CNS) but has also been detected in lung, kidney, and liver. In contrast, CB2 is primarily expressed on immune cells such as B-cells, T-cells, macrophages, dendritic cells, and microglia (Pacher and Mechoulam, 2011). Thus, the ECS affects both immune responses and cognition. In addition, studies have suggested that CB2 plays a role in the modulation of microglia activity relevant to AD.

A.-C.S. and R.L. contributed equally and J.A. and A.Z. shared senior authorship.

\* Corresponding author at: Institute for Molecular Psychiatry, University of Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany. Tel.: +49 (0)228 6885 300; fax: +49 (0)228 6885 301.

E-mail address: [a.zimmer@uni-bonn.de](mailto:a.zimmer@uni-bonn.de) (A. Zimmer).

Early studies already suggested a crucial role for the CB2 receptor in AD, based on findings that CB2 is overexpressed in A $\beta$  plaque-associated microglia (Benito et al., 2003). Up-regulation of CB2 expression by microglia has also been described in Huntington disease, simian immunodeficiency virus-induced encephalitis, human immunodeficiency virus (HIV) encephalitis, and multiple sclerosis (Benito et al., 2003, 2005, 2008; Ramírez et al., 2005).

To date however, most studies have examined the effects of pharmacological modulation of the ECS, showing that stimulation with specific CB2 agonists reduces the A $\beta$  burden and neuroinflammation and rescues cognitive deficits in AD mouse models (Aso et al., 2013). Similar observations were also made in rats injected with amyloid fibrils in the hippocampal CA1 region to mimic AD pathology, followed by treatment with a CB2 agonist (Wu et al., 2013). In addition, extended oral treatment with cannabinoids reduced A $\beta$  levels of APP2576 mice (Martín-Moreno et al., 2012) and decreased A $\beta$  plaque-deposition in 5xFAD APP transgenic mice (Chen et al., 2013). However, since the precise involvement of cannabinoid receptors remains unknown, we investigated here CB2 in AD using *in vitro* and *in vivo* models. Our results indicate an important function of CB2 in the modulation of AD-associated neuroinflammation.

## 2. Methods

### 2.1. Microglia cultures

Primary neonatal microglia were generated from 1- to 5-day-old C57BL/6J (CB2<sup>+/+</sup>) and CB2<sup>-/-</sup> pups (Buckley et al., 2000), according to the protocol from Saura et al. (Saura et al., 2003). Briefly, after cervical dislocation, extracted brains were collected in ice-cold phosphate-buffered saline (PBS). Cortices of both hemispheres were collected after removal of meninges and triturated until a single-cell suspension was achieved. Isolated cells were cultivated as mixed glia culture until they reached confluency at approximately 21 days. Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) high-glucose (Gibco, Darmstadt, Germany) containing 10% fetal calf serum (FCS) (PAA, Freiburg, Germany), 1% penicillin/streptomycin (Gibco), 1% MEM/NEAA (Gibco), and 0.001%  $\beta$ -mercaptoethanol (Gibco), medium was changed twice weekly. Microglia cells were harvested by mild trypsinization and re-seeded in 24-well-plates for stimulation experiments or phagocytosis assay at a density of  $1.5 \times 10^5$  cells/mL. After re-seeding, cells rested for 24 hours.

For stimulation, cells were treated for 16 hours with LPS (100 ng/mL) (Sigma-Aldrich, Taufkirchen, Germany) and interferon- $\gamma$  (IFN $\gamma$ ; 20 ng/mL) (R&D Systems, Wiesbaden, Germany) or for 48 hours with interleukin-4 (IL-4; 100 U/mL) (eBioscience, Frankfurt, Germany).

### 2.2. Phagocytosis assay

Microglia cells were incubated with fluorescently labeled A $\beta$  42 (Alexa-Flour 649) for 1 hour at room temperature, or at 4 °C as a negative control for unspecific binding. Labeling of A $\beta$  was performed by using DyLight™649 Microscale Antibody Labeling Kit (ThermoScientific, Waltham, MA). After washing, cells were stained with CD11b antibodies for flow cytometry.

### 2.3. Animals

APP/PS1 mice were purchased from Charles River Laboratories (B6.Cg-Tg (APP<sup>swe</sup>(K594 N/M595 L)/PSEN1<sup>dE9</sup>)85Dbo/J; Charles River Laboratories, Germany GmbH) and crossed with CB2<sup>-/-</sup> mice (Buckley et al., 2000). APP/PS1\*CB2<sup>+/-</sup> mice were then crossed with

CB2<sup>-/-</sup> mice. All animals were bred and housed in a specific pathogen-free animal facility under standard animal housing conditions in a 12h dark-light cycle with access to food and water *ad libitum* according to German guidelines for animal care. APP/PS1\*CB2<sup>-/-</sup> pups were born at a less-than-expected ratio. We thus crossed APP/PS1\*CB2<sup>-/-</sup> mice with CB2<sup>-/-</sup> mice. The experiments were carried out with mice at the age of 9 and 14 months from both breeding strategies. Mice were genotyped by polymerase chain reaction (PCR) using DNA from tail tissue. Primers: CB2 common (5'-GTC GAC TCC AAC GCT ATC TTC-3'), CB2 wild-type (5'-GTG CTG GGC AGC AGA GCG AAT C-3'), CB2 knock-out (5'-AGC GCA TGC AGA CTG CCT-3'), PSEN1-F (5'-GGT CCA CTT CGT ATG CTG-3'), and PSEN-R (5'-AAA CAA GCC CAA AGG TGA T-3').

Experimental procedures complied with all regulations for animal experimentation in Germany and were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz in Nordrhein-Westfalen, Germany (AZ 87-51.04.2011.A041, 8.84-02.05.20.11.101).

### 2.4. Isolation of intracerebral mononuclear cells

Brains were removed after intracardial perfusion with ice-cold PBS and minced with small scissors followed by trituration with a 20-gauge needle. Brain fragments were incubated with 1 mL collagenase solution (Roche, Mannheim, Germany) (1 mg/mL) for 45 minutes at 37 °C. Subsequently, tissue fragments were triturated with 1 mL DNase solution (Roche) (1 mg/mL) and incubated for 45 minutes at 37 °C. After establishing a single-cell suspension, cells were washed and re-suspended in 70% percoll solution (GE Healthcare, Uppsala, Sweden) and overlaid with a 30% percoll solution before spinning down for 25 minutes without a break. Mononuclear cells were collected at the interphase, washed with PBS, and, after centrifugation at 1800 rpm for 10 minutes at 4 °C, subjected to cell surface marker staining for flow cytometry.

### 2.5. Flow cytometry

Cells were labeled with fluorochrome-coupled anti-mouse antibodies or biotin-coupled anti-mouse antibodies, followed by incubation with streptavidin-fluorochrome coupled secondary reagents. Antibodies (RRID): CD11 b (AB\_469343), CD11 b (AB\_394773), CD11 b (AB\_1582236), CD11 b (AB\_312789), CD11 b (AB\_465549), CD40 (AB\_465651), CD16/32 (AB\_312801), MMR (CD206) (AB\_2144899), ICAM (CD54) (AB\_465095). Immunofluorescence of labeled cells was subsequently measured by using a FACS Canto II (BD Bioscience, Heidelberg, Germany), equipped with FACSDiva software. Data analysis was performed using FlowJo software (Tree Star Inc, Ashland, OR).

### 2.6. Total RNA preparation

RNA was extracted by TRIzol extraction protocol. Briefly, frozen tissue was homogenized in 1 mL or 800  $\mu$ L TRIzol (Invitrogen, Camarillo, CA). After precipitation with isopropanol, RNA was stored at -80 °C. For cDNA synthesis, 400 ng RNA was incubated for 5 minutes at 65 °C and then reverse transcribed at 42 °C for 50 minutes. A total volume of 20  $\mu$ L included 4  $\mu$ L first-strand buffer (Invitrogen), 2  $\mu$ L 0.1 mo/L DTT, 1  $\mu$ L 10 mmol/L dNTPs, 1  $\mu$ L oligo(dT)20 primer (Invitrogen), and 200 U SuperScript II reverse transcriptase (Invitrogen).

### 2.7. Quantitative RT-PCR

RT-quantitative PCR of cDNA samples was performed using ABI 7900 sequence detector (Perkin-Elmer, Waltham, MA) and

متن کامل مقاله

دریافت فوری ←

**ISI**Articles

مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات