Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Cytokine-induced sleep: Neurons respond to TNF with production of chemokines and increased expression of *Homer1a* in vitro

Maureen Karrer [a], Martin Alexander Lopez [a], Daniel Meier [a], Cyril Mikhail [b], Omolara O. Ogunshola [c], Andreas Müller [a], Laura Strauss [a], Mehdi Tafti [b,1], Adriano Fontana [a,1,*]

[a] Institute of Experimental Immunology, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland
[b] Center for Integrative Genomics (CIG), University of Lausanne, 1015 Lausanne, Switzerland
[c] Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form 17 November 2014
Accepted 18 November 2014
Available online xxxx

Keywords:
Sickness behavior
Depression
Multiple sclerosis
Rheumatoid arthritis
Inflammatory bowel disease
Neuroinflammation

ABSTRACT

Interactions of neurons with microglia may play a dominant role in sleep regulation. TNF may exert its somnogeneic effects by promoting attraction of microglia and their processes to the vicinity of dendrites and synapses. We found TNF to stimulate neurons (i) to produce CCL2, CCL7 and CXCL10, chemokines acting on mononuclear phagocytes and (ii) to stimulate the expression of the macrophage colony stimulating factor (*M-CSF/Csf1*), which leads to elongation of microglia processes. TNF may also act on neurons by affecting the expression of genes essential in sleep–wake behavior. The neuronal expression of *Homer1a* mRNA, increases during spontaneous and enforced periods of wakefulness. Mice with a deletion of *Homer1a* show a reduced wakefulness with increased non-rapid eye movement (NREM) sleep during the dark period. Recently the TNF-dependent increase of NREM sleep in the dark period of mice with CD40-induced immune activation was found to be associated with decreased expression of *Homer1a*. In the present study we investigated the effects of TNF and IL-1β on gene expression in cultures of the neuronal cell line HT22 and cortical neurons. TNF slightly increased the expression of *Homer1a* and IL-1β profoundly enhanced the expression of *Early growth response 2* (*Egr2*). The data presented here indicate that the decreased expression of *Homer1a*, which was found in the dark period of mice with CD40-induced increase of NREM sleep is not due to inhibitory effects of TNF and IL-1β on the expression of *Homer1a in neurons*.

1. Introduction

Patients with autoimmune diseases suffer from sickness behavior syndrome (SBS), which is characterized by fatigue, malaise, decreased appetite, weight loss, and reduced social activities (Dantzer et al., 2008). A causal link between tumor necrosis factor (TNF) and SBS is suggested because treatment with the soluble TNF receptor p75 or with antibodies against TNF improved fatigue and depression in patients with rheumatoid arthritis (RA), psoriasis, and Crohn's disease (Farahani et al., 2006; Katz et al., 2009; Lichtenstein et al., 2002; Moreland et al., 2006; Taylor and Feldmann, 2009; Tyring et al., 2006). In recent studies on experimental SBS in mice, the immune activation was triggered by anti-CD40 monoclonal antibodies (mAb), which activate the CD40 receptor in B cells and antigen presenting cells including macrophages and dendritic cells. Mice treated with CD40 mAb show a decrease in wakefulness and an increase in non-rapid eye movement (NREM) sleep during the dark period (Gast et al., 2013; Taraborrelli et al., 2011). Inactivation of the TNF receptor 1 gene or treatment with soluble TNF receptor p75 protects mice from CD40 mediated sleep–wake changes, but not (Nimmerjahn et al., 2005) from immune activation (Gast et al., 2013; Taraborrelli et al., 2011).

Abbreviations: CD40L, CD40 ligand; CD40 mAb, anti-CD40 monoclonal antibody; *Egr2*, early growth response 2; *Fosl2*, Fos-like antigen 2; *Homer1a* KO mice, *Homer1a* gene knockout mice; *Jph3*, junctophilin 3; IL-, interleukin-; *M-CSF/Csf1*, macrophage colony stimulating facto5; *Nptx2*, neuronal pentraxin 2; NREM, non-rapid eye movement sleep; *Ptgs2*, prostaglandin-endoperoxide synthase 2; SBS, sickness behavior syndrome; SD, sleep deprivation; siRNA, short interfering RNAs; SSctx, primary somatosensory cortex; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1.

* Corresponding author at: Institute of Experimental Immunology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. Tel.: +41 44 635 37 19; fax: +41 44 635 68 83.
E-mail address: fontana.adriano7@gmail.com (A. Fontana).
[1] These authors share last authorship.

http://dx.doi.org/10.1016/j.bbi.2014.11.008
0889-1591/

Reciprocal signaling between neurons and microglia may be essential in remodeling of brain circuits including the formation, modification, and elimination of synaptic structures. This concept is supported by evidence that microglia processes periodically contact dendritic spines and axon terminals in vivo (Davalos et al., 2005; Nimmerjahn et al., 2005; Wake et al., 2009). Contacts between neurons and microglia foster cell–cell communication via membrane receptors and soluble mediators including cytokines. Among the latter TNF, is considered to play an important role in behavior and immune-mediated inflammation. In inflammatory diseases TNF is produced mainly by microglia and macrophages. Examples are experimental autoimmune encephalomyelitis and murine cerebral malaria and (Medana et al., 1997; Renno et al., 1995). As shown by *in situ* hybridization the type of cell producing TNF in rats injected intravenously with lipopolysaccharide (LPS) was identified as microglia, but not as neurons and astrocytes (Buttini et al., 1997). Using *in situ* hybridization and immunohistochemistry microglia and macrophages were characterized as the major sources of TNF in middle cerebral artery occlusion in mice (Gregersen et al., 2000). However, immunohistochemical studies show that the physiological role of TNF in sleep regulation may be mediated by TNF production in neurons (Churchill et al., 2008). TNF binds to TNF receptor TNFR1 and TNFR2, both of which are expressed on neurons (Marchetti et al., 2004). The production of TNF is strongly influenced by circadian rhythms. Levels of *Tnf* mRNA and TNF correlate with sleep propensity, that is, high sleep propensity is associated with high levels of *Tnf* mRNA and TNF. Furthermore increased TNF is seen during sleep deprivation (Imeri and Opp, 2009; Kaushal et al., 2012; Krueger, 2008). Central or systemic injections of TNF or IL-1β increase the duration of NREM sleep and the EEG delta power, the latter being an index of sleep intensity (Krueger, 2008; Opp, 2005). In this regard, it is also noteworthy that TNF and IL-1β cause dysregulation of Clock genes with decreased expression of the period genes *Per1*, *Per2* and *Per3* and the PARbZip transcription factors including *Dbp*, *Tef* and *Hlf* (Cavadini et al., 2007).

The regulation of sleep has been studied intensively using sleep deprivation (SD). SD leads to increased expression of, e.g., immediate early genes/transcription factors, mitochondrial genes, genes involved in energy metabolism, and neurotransmitter transporters and receptors (Cirelli, 2009). Transcriptome profiling in inbred mouse strains showed that genetic background affects susceptibility to SD at the transcriptional level. When taking the genetic background into account, expression of *Homer1a* associates with changes in homeostatic sleep need (Franken et al., 2001; Mackiewicz et al., 2008; Maret et al., 2007) Neurons expressing *Homer1a* also express early growth response 2 (*Egr2*/*Krox20*), *Fos*-like antigen 2 (*Fosl2*), prostaglandin-endoperoxide synthase 2 (*Ptgs2*), junctophilin 3 (*Jph3*), and neuronal pentraxin 2 (*Nptx2*) (Maret et al., 2007). Expression of these transcripts equally increases with sleep need. *Homer1a* mRNA increases during spontaneous and enforced periods of wakefulness (Huber et al., 2007; Maret et al., 2007; Nelson et al., 2004). Mice with a deletion of *Homer1a* (*Homer1a* KO mice) show a reduced wakefulness with increased NREM sleep during the dark period (Naidoo et al., 2014, 2012). Previous studies of mice with CD40-induced sleep showed that *Homer1a* expression decreases during the second half of the dark period when mice show increased NREM sleep (Gast et al., 2013). In the cortex of CD40 mAb-treated mice the decline of *Homer1a* in the dark period was associated with a significant depression of *Egr2*, *Nptx2*, and *Fosl2*.

The effects of cytokines on sleep–wake behavior may involve communications of neurons with microglia. Cytokines may regulate intercellular interactions by promoting neurons to produce chemokines, which attract microglia to neuronal dendrites and synapses. In this study, we investigated whether TNF may lead to the expression of chemokines by neurons. The data presented here show that TNF activates neuronal production of CCL2, CCL7 and CXCL10 and of *M-CSF*/*CSF-1*. These chemokines have been well-described to attract mononuclear phagocytes and to lead to the extension of microglia processes. By influencing synaptic strength and by secreting mediators such as glutamate and prostaglandins, TNF-stimulated microglia cells may play a pivotal role in the regulation of sleep. Our study on effects of cytokines on neurons also addresses the question as to weather downregulation of *Homer1a* expression in CD40-activated mice is due to cytokine-mediated repression of *Homer1a*. Such effects would induce transitions between wakefulness and sleep. We found that HT22 cells and cortical neurons respond to TNF and IL-1β with increased *Egr2* and *Ptgs2* expression, but not with a downregulation of *Homer1a*. Thus in CD40 mAb-treated mice, the decrease of *Homer1a* expression in the dark period is not due to direct inhibitory effects of TNF and IL-1β on *Homer1a* transcription.

2. Methods

2.1. Primary cortical neurons, HT22 cells and cytokine treatment

Neurons were isolated from the cerebral cortex of C57Bl/6 J mice gestational stage E14–E18 as previously described (Ogunshola et al., 2002). Dissected cortices were dissociated in Hank's buffered salt solution containing trypsin or papain and DNase I for 5 min at 37 °C. Neurons were seeded on poly-L-lysine coated petri dishes (3×10^6 cells per 100 mm dish) in Neurobasal medium containing B27 supplement (1×), albumax (0.25 g/ml), 1% sodium pyruvate, 100 U/ml penicillin–streptomycin and 1 mm L-glutamine (GIBCO, Invitrogen, AG, Switzerland). The cultures (purity 98%), were maintained for 17 days at normal atmosphere (21% O_2) in a humidified incubator at 37 °C. For analysis of cytokine expression cells were treated on day 14–17 with TNF (Peprotech, London UK) or PBS control for 4–8 h. Thereafter cells and supernatants were harvested.

For expression of *Homer1a*, *Egr2*, *Ptgs2* and *Fosl2* primary cortical neurons were plated either on 35 mm dishes (at 1.5×10^6 cells; density = 1560 cells/mm^2) pre-coated with 0.1 mg/ml poly-L-lysine. Cultures were maintained in a humidified CO_2 incubator (5% CO_2, 37 °C) and half of the medium was changed once a week. After treatment of cortical neurons with cytokines or PBS control during 4 h, total RNA from cell cultures was extracted.

The mouse hippocampal neuronal cell line HT22 was obtained from David Schubert at the Salk Institute (La Jolla, CA). HT22 cells were plated in 12-well tissue cultures plates (100,000 cells per well) in DMEM with 10% FCS. Two days after plating, cultures were serum deprived for 1 h. Thereafter, HT22 cells were treated with TNF or IL-1β for 4 h and RNA extracted.

2.2. RNA extraction and quantification

RNA was extracted using RNA easy mini kit (Qiagen). All RNA samples were DNase-treated and quantified on a NanoDrop ND-1000 spectrophotometer. To quantify the RNA expression level, 1 μg of RNA was reverse-transcribed in 20 μl using random hexamers and Superscript II reverse transcriptase (Invitrogen) according to standard procedures. The cDNA was diluted 10 times and 2 μl were amplified in a 10 μl TaqMan reaction on ABI PRISM HT 7900 detection system in technical triplicate. Cycler conditions were 50 °C 2 min, 95 °C 10 min and 45 cycles at 95 °C 15 s and 60 °C 1 min. Forward primer, reverse primer, and probe sequences are given in Table 1. The gene expression level was normalized to three reference genes (*Rsp9*, *TBP*, and *EEF1a1*) using Qbase software (Hellemans et al., 2007). The fold changes indicative of the relative

Please cite this article in press as: Karrer, M., et al. Cytokine-induced sleep: Neurons respond to TNF with production of chemokines and increased expression of *Homer1a* in vitro. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.11.008