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CD40 activation induces NREM sleep and modulates genes associated with sleep homeostasis

Heidemarie Gast [a], Andreas Müller [b], Martin Lopez [b], Daniel Meier [b], Reto Huber [c], Frieder Dechent [d,e], Marco Prinz [d,e], Yann Emmenegger [f], Paul Franken [f], Thomas Birchler [b,1], Adriano Fontana [b,*,1]

[a] Department of Neurology, Inselspital, University Hospital Berne, University of Bern, Bern, Switzerland
[b] Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland
[c] University Children's Hospital Zurich, Zurich, Switzerland
[d] Department of Neuropathology, University of Freiburg, Freiburg, Germany
[e] BIOSS Center for Biological Signaling, University of Freiburg, Freiburg, Germany
[f] Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

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ABSTRACT

The T-cell derived cytokine CD40 ligand is overexpressed in patients with autoimmune diseases. Through activation of its receptor, CD40 ligand leads to a tumor necrosis factor (TNF) receptor 1 (TNFR1) dependent impairment of locomotor activity in mice. Here we report that this effect is explained through a promotion of sleep, which was specific to non-rapid eye movement (NREM) sleep while REM sleep was suppressed. The increase in NREM sleep was accompanied by a decrease in EEG delta power during NREM sleep and by a decrease in the expression of transcripts in the cerebral cortex known to be associated with homeostatic sleep drive, such as *Homer1a*, Early growth response 2, Neuronal pentraxin 2, and Fos-like antigen 2. The effect of CD40 activation was mimicked by peripheral TNF injection and prevented by the TNF blocker etanercept. Our study indicates that sleep–wake dysregulation in autoimmune diseases may result from CD40 induced TNF:TNFR1 mediated alterations of molecular pathways, which regulate sleep–wake behavior.

1. Introduction

In autoimmune diseases the destruction of target cells in various organs is caused by the action of anti-self T-cells, autoantibodies and activated macrophages. Besides organ dysfunction, however, patients also suffer from sickness behavior syndrome (SBS), which is characterized by fatigue, malaise, decreased appetite, weight loss and reduced social activities (Dantzer et al., 2008). In multiple sclerosis, autoimmune hepatitis, rheumatoid arthritis, systemic lupus erythematosus (SLE), and inflammatory bowel disease patients suffer from fatigue that is a significant comorbidity severely affecting quality of life. SBS is also frequently seen in acute or chronic infections and has been assumed to be due to the action of cytokines (Krueger, 2008; Opp, 2005). When administered peripherally or directly into the cerebral ventricles, cytokines including TNF, interleukin (IL)-1β, IL-6, and interferons induce SBS. Likewise SBS, which follows activation of Toll-like receptor (TLR) 3 with synthetic double-stranded RNA (polyI:C) or stimulation of TLR4 with lipopolysaccharide (LPS), is associated with an increase in cytokine production both in the central nervous system (CNS) and in the immune compartment (Bluthe et al., 2000; Cunningham et al., 2007).

To study the mechanism of SBS in autoimmune diseases we have developed a new animal model using the CD40–CD40 ligand (CD40L) pathway of immune activation. CD40 is expressed mainly

Abbreviations: BL, baseline activity; BBB, blood brain barrier; CD40L, CD40 ligand; CD40 mAb, anti-CD40 monoclonal antibody; CNS, central nervous system; Egr2, early growth response 2; Eta, etanercept; Fosl2, Fos-like antigen 2; Homer1a KO mice, homer1a gene knockout mice; Jph3, junctophilin 3; IL-, interleukin-; i.p., intraperitoneally; L/D, light-dark; LPS, lipopolysaccharide; nBA, number of brief awakenings; Nptx2, neuronal pentraxin 2; OSA, obstructive sleep apnea; NREM, non-rapid eye movement; REM, rapid eye movement; Ptgs2, prostaglandin-endoperoxide synthase 2; SBS, sickness behavior syndrome; SCN, suprachiasmatic nucleus; SD, sleep deprivation; siRNA, short interfering RNAs; SLE, systemic lupus erythematosus; SSctx, primary somatosensory cortex; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; ZT, Zeitgeber time.

* Corresponding author. Address: 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: adriano.fontana@uzh.ch (A. Fontana).
[1] These authors share last authorship.

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on antigen presenting cells, B-lymphocytes and endothelial cells (for review: Grewal and Flavell, 1998). CD40 mediated signaling is induced by CD40L, which is expressed by activated $CD4^+$ T-cells. CD40 activation leads to production of cytokines including TNF, IL-1β, IL-6, IL-8, interferon α, β and γ, IL-12, IL-17, IL-23, and granulocyte-macrophage colony stimulating factor (Danese et al., 2004; Elgueta et al., 2009). CD40L:CD40 interaction is essential for the autoimmune response to self antigens. It was demonstrated that inhibition of CD40 or CD40L renders mice resistant to the induction of experimental autoimmune diseases (for review: Peters et al., 2009). On the other hand increased signaling via CD40L:CD40 has been observed in multiple sclerosis, rheumatoid arthritis, SLE, and in HIV infected patients, diseases that are well known to be associated with fatigue (Sui et al., 2007; Peters et al., 2009). SBS is also induced in patients with chronic lymphatic leukemia or multiple myeloma when treated with monoclonal antibodies (mAb) to CD40 (Advani et al., 2009; Furman et al., 2010; Hussein et al., 2010). Our previous studies in mice show that activation of CD40 leads to SBS (Taraborrelli et al., 2011). The latter is characterized by weight loss and reduced locomotor activity. Interestingly, mice with an inactivation of the TNF receptor 1 gene (*Tnfr1*$^{-/-}$) are completely protected from CD40 mediated SBS, but not from immune activation (Taraborrelli et al., 2011). This study made use of a monoclonal antibody to CD40 (CD40 mAb), which by binding to this cell surface receptor molecule activates intracellular signaling pathways and thereby activates macrophages to produce cytokines (Fig. 1). Injection of the CD40 mAb into mice mimics the effects seen in transgenic mice overexpressing CD40L. Activation of CD40 by CD40L or by CD40 mAb leads to a wasting syndrome with inflammation and hypertrophy of lymphoid tissues as characterized by loss of normal follicular structure and co-localization of activated T cells and B cells in large lymphocytic clusters. Lymphadenopathy and splenomegaly is also due to myeloid hyperplasia. Moreover pronounced infiltrates of B cells, NK cells, CD4 T cells, dendritic cells and macrophages are seen in various organs including lung, liver, pancreas and gastrointestinal tract (Clegg et al., 1997; Kimura et al., 2006).

There is substantial evidence that TNF and IL-1β enhance non-rapid eye movement (NREM) sleep when injected into animals (Krueger, 2008; Opp, 2005; Shoham et al., 1987). Moreover in rats *Tnf* mRNA brain levels vary with sleep propensity (Bredow et al., 1997; Floyd and Krueger, 1997). Furthermore, reduction of *Tnf* mRNA by microinjection of *Tnf* short interfering RNA (siRNA) into the rat primary somatosensory cortex (SSctx) reduced EEG delta power during NREM sleep at the site of injection, but not contralaterally (Taishi et al., 2007). EEG delta power (1–4 Hz) is widely used as a state variable of the sleep homeostatic process (Borbely and Achermann, 1999). Local application of TNF onto the SSctx induces EEG asymmetry with increased activity in the delta frequencies during NREM sleep, while the duration of NREM did not change (Yoshida et al., 2004). The response to sleep deprivation (SD) has also been found to involve TNF. Pretreatment of rabbits with fragments of human recombinant soluble TNF receptor 1 and of human type I IL-1 receptor attenuated the sleep rebound after SD (Takahashi et al., 1996a,b). Taken collectively TNF may not only be involved in SBS, but is likely to also play a role in physiological sleep regulation (Krueger, 2008). At the molecular level the homeostatic regulation of sleep has been studied intensively using 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 show 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; Maret et al., 2007; Mackiewicz et al., 2008). 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).

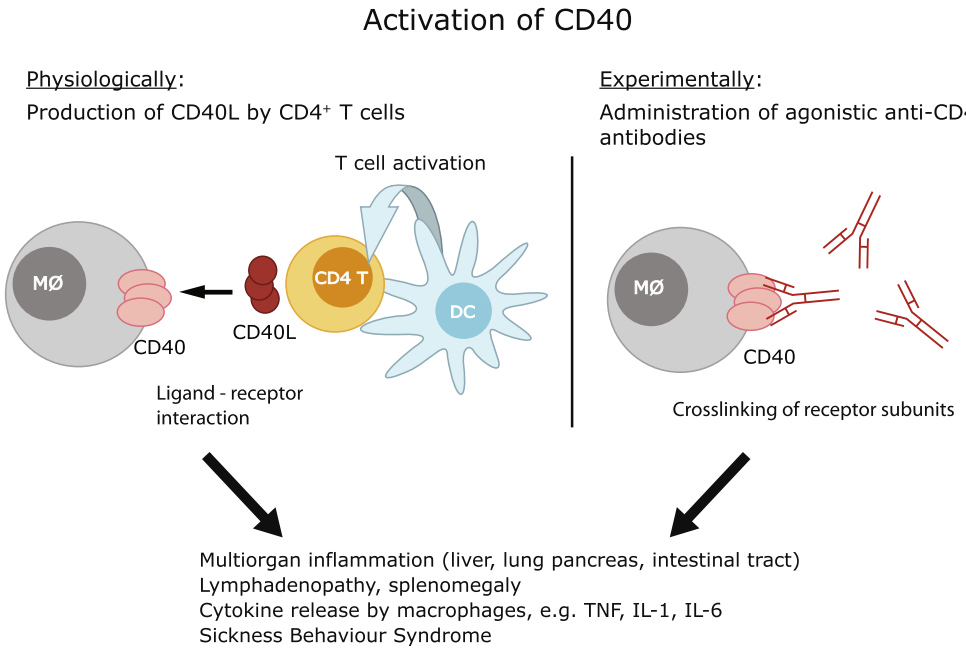

Fig. 1. Activation of CD40 by CD40 ligand or agonistic anti-CD40 antibodies leads to multiorgan inflammation and sickness behavior syndrome. Antigen presentation by dendritic cells to MHC class II restricted CD4 T cells induces the production of CD40L, which bind as homotrimers to CD40 expressed on macrophages and dendritic cells. Binding of CD40L–CD40 molecules, which are also expressed in trimeric forms, activates the cells as evidenced by induction of migration, upregulation of MHC class II and adhesion molecules as well as production of chemokines and cytokines. Among the different anti-CD40 monoclonal antibodies available, there are some which after binding to CD40 induce intracellular signaling and thereby mimic the effect of CD40L. Both CD40L and the agonistic CD40 mAb promote multiorgan inflammation and sickness behavior syndrome.