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The transcription factor nuclear factor interleukin 6 mediates pro- and anti-inflammatory responses during LPS-induced systemic inflammation in mice

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ABSTRACT

The transcription factor nuclear factor interleukin 6 (NF-IL6) plays a pivotal role in neuroinflammation and, as we previously suggested, hypothalamus–pituitary–adrenal-axis-activation. Here, we investigated its contribution to immune-to-brain communication and brain controlled sickness symptoms during lipopolysaccharide (LPS)-induced (50 or 2500 μg/kg i.p.) systemic inflammation in NF-IL6-deficient (KO) or wildtype mice (WT). In WT LPS induced a dose-dependent febrile response and reduction of locomotor activity. While KO developed a normal fever after low-dose LPS-injection the febrile response was almost abolished 3–7 h after a high LPS-dose. High-dose LPS-stimulation was accompanied by decreased (8 h) followed by enhanced (24 h) inflammation in KO compared to WT e.g. hypothalamic mRNA-expression including microsomal prostaglandin E synthase, inducible nitric oxide synthase and further inflammatory mediators, neutrophil recruitment to the brain as well as plasma levels of inflammatory markers such as IL-6 and IL-10. Interestingly, KO showed reduced locomotor activity even under basal conditions, but enhanced locomotor activity to novel environment stress. Hypothalamic–pituitary–adrenal-axis-activity of KO was intact, but tryptophan-metabolizing enzymes were shifted to enhanced serotonin production and reuptake. Overall, we showed for the first time that NF-IL6 plays a dual role for sickness response and immune-to-brain communication: acting pro-inflammatory at 8 h but anti-inflammatory at 24 h after onset of the inflammatory response reflecting active natural programming of inflammation. Moreover, reduced locomotor activity observed in KO might be due to altered tryptophan metabolism and serotonin reuptake suggesting some role for NF-IL6 as therapeutic target for depressive disorders.

1. Introduction

Systemic inflammation, experimentally induced by the bacterial inflammatory compound lipopolysaccharide (LPS), leads to brain-controlled sickness symptoms including fever. Underlying immune-to-brain communication is mediated by three major signaling pathways: a humoral [I] (Roth et al., 2009; Roth and Blatteis, 2014), a cellular [II] (Rummel et al., 2010; Aguliar-Valles et al., 2014; Dantzer et al., 2008; Serrats et al., 2010) and a neural [III] one (Roth and Blatteis, 2014). (I) LPS induces a peripheral release of proinflammatory cytokines like interleukin(IL)-6, which in addition to LPS itself act as humoral mediators on cells of the blood–brain-barrier (BBB), namely on brain endothelial cells (Eskilsson et al., 2014; Wilhelms et al., 2014; Ching et al., 2007), or brain structures with a leaky BBB such as the sensory circumventricular organs (Roth et al., 2004; Ott et al., 2010; Wuchert et al., 2008, 2009). Subsequently, hypothalamic nuclei are activated via neuronal projections or secondary mediators produced locally at the site of action (Capuron and Miller, 2011). (II) Moreover, peripheral cytokines and LPS stimulate immune cells to migrate to the brain, locally release cytokines and, thereby, contribute to brain cell activation and behavioral impairments (Aguliar-Valles et al., 2014; D'Mello et al., 2009; McColl et al., 2007). The relevance of this pathway for the manifestation of fever

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and other rapidly developing sickness symptoms has not yet been established. (III) Furthermore, LPS can activate vagal and other sensory afferent nerves, which may contribute to sickness behavior (Dantzer et al., 2008) and to the development of the early stage of fever via a rapid peripheral formation of PGE2 (Roth and Blatteis, 2014; Steiner et al., 2006). The neural pathway is not analyzed in this study.

LPS induces a spatiotemporal genomic activation of brain cells. First, there is early nuclear translocation of the pivotal transcription factor nuclear factor (NF) κB, followed by signal transducer and activator of transcription (STAT)3 and, with some delay, NF-IL6, which peaks at 8 h (Damm et al., 2011; Rummel et al., 2005; Nadjar et al., 2003; Gautron et al., 2002). These transcription factors in turn regulate the expression of target genes responsible for the development of the sickness response.

Recently, we revealed that NF-IL6 (C/EBPβ) is activated in the brain during the late phase of systemic inflammation after both, low and high dose LPS-stimulation (Damm et al., 2011; Harden et al., 2014) and we hypothesized that NF-IL6 might be involved in either the maintenance or the termination of the febrile response (Damm et al., 2011). Furthermore, we obtained evidence that NF-IL6 might be implicated in the control of hypothalamic–pituitary–adrenal-(HPA)axis-activity as it is activated in response to exposure to a novel environment, an established psychological stressor (Fuchs et al., 2013). The HPA-axis is of special interest because it acts as negative feedback mechanism on immune-to-brain communication (Haddad et al., 2002) and it influences the serotonin system (Leonard, 2005), which is the key for the development of depressive disorders (Myint and Kim, 2014; Badawy, 2013). It is also known that NF-IL6 influences the prostaglandin E_2 (PGE2) synthesis by controlling one of the rate limiting enzymes, namely microsomal prostaglandin E synthase (mPGES) (Uematsu et al., 2002). In addition, it is associated with memory and learning (Taubenfeld et al., 2001), LPS-induced neuronal death (Ramji and Foka, 2002; Pan et al., 2013; Pena-Altamira et al., 2014), excitotoxic brain injury (Cortes-Canteli et al., 2008) and post-ischemic neuroinflammation and brain damage (Halterman et al., 2008; Kapadia et al., 2006). In that context, NF-IL6 is involved in several diseases that are accompanied by increased brain inflammation like, for example, Alzheimer's disease (Strohmeyer et al., 2014). Overall, NF-IL6 seems to be important for immune-to-brain communication, brain inflammation and the development of sickness responses. The exact contribution of NF-IL6, however, still remains to be determined.

Thus, the goal of the present study was to clarify for the first time the role of NF-IL6 for humoral and cellular immune-to-brain signaling pathways, for the strength of LPS-induced systemic and brain-intrinsic inflammatory responses, and especially for the manifestation of fever. Therefore, we stimulated NF-IL6-deficient mice with LPS, monitored the sickness response, analyzed critical signaling molecules including markers of inflammation, the serotonin system and conducted measures of HPA-axis responsiveness such as novel environment stress.

2. Materials and methods

2.1. Animals

C/EBPβ$^{-/-}$ (KO) and C/EBPβ$^{+/+}$ (WT) litter mates were generated in an in-house breeding from heterozygous (C/EBPβ$^{-/+}$) parental animals obtained from The Jackson Laboratory (Bar Harbor, ME, USA; stock number 006873). Generation of C/EBPβ$^{-/-}$ mice and their genetically background was previously described by Screpanti et al. (1995). Genotyping was performed according the protocol provided by The Jackson Laboratory (http://jaxmice.jax.org/protocolsdb/, stock number 006873). During the experiment 6–12 weeks old male and female animals were individually housed with constant access to water and powdered standard lab chow (Ssniff Spezialdiäten GmbH, Soest, Germany) in a climatic chamber (Weiss Umwelttechnik GmbH, Typ 10′US/+5 – + 40 DU, Germany) at an ambient temperature of 31 °C and 50% humidity at a 12:12 h light–dark cycle (lights on at 07:00). Animals were implanted with intra-abdominal radio transmitters for measurement of core body temperature (Tb) and motor activity (MiniMitter Company Inc., Sunriver, OR, USA). Implantation of transmitters was performed about 1 week before the experiments using a mixture of ketamine (approximately 4 mg/animal; Medistar Arzneimittelvertrieb GmbH, Ascheberg, Germany) and xylazine (approximately 0.2 mg/animal; CP-Pharma, Burgdorf, Germany) as anesthetic (intraperitoneally, i.p.). Meloxicam (1 mg/kg body weight, BW; Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany) was used for surgical analgetic treatment. An automatic data acquisition system was used (VitalView, Respironics Inc-MiniMitter, Bend, OR, USA). For habituation, mice were handled at least 3 days before experiments. All animal procedures were conducted according to the guidelines approved by the local ethics committee (ethics approval number GI 18/2 – 51/2008).

2.2. Treatment and experimental protocols

The NF-IL6 deficient mice showed, at the same age, lower body weight than their WT littermates (at the beginning of experiments: KO 21.16 ± 0.73 g; WT 26.01 ± 0.57 g). To account for these changes LPS doses were adapted to body weight. When mice were switched from the conventional cages after at least one week of recovery of the surgery to new, experimental cages Tb and motor activity were recorded for the first 4 h (10:00–14:00). These data were used as readouts for novel environment stress (NES; $n = 47$). For inflammatory stress experiments, mice were i.p. injected with LPS (2500 or 50 μg/kg BW; derived from Escherichia coli, serotype 0111:B4, Sigma Chemicals, Deisenhofen, Germany) diluted in sterile pyrogen-free 0.9% phosphate buffered saline (PBS; Dulbecco's Phosphate Buffered saline, PAA, Cölbe, Germany) at a total injection volume of 5 ml/kg BW. The dose of LPS was chosen according to previous studies showing either (~50 μg/kg) a moderate febrile response (Rudaya et al., 2005) and transcription factor activation in the brain (Rummel et al., 2008) or (2.5 mg/kg) robust systemic and brain inflammatory response accompanied by a strong fever (Rudaya et al., 2005) and recruitment of neutrophil granulocytes to the brain (Aguliar-Valles et al., 2014; Rummel et al., 2010). Another previous report showed abolished fever in NFκB deficient animals to an LPS-stimulation of 50 μg/kg (Kozak et al., 2006). Here, we wanted to reveal the role for NF-IL6 for the same type of febrile response, brain as well as peripheral inflammation and underlying humoral and cellular immune-to-brain signaling pathways. Controls were injected with an equal volume of sterile pyrogen-free 0.9% PBS. All injections were performed between 9:00 and 10:30. To reduce the number of animals used for the experiments; animals received either the low dose of LPS and were treated with PBS one week later or injected with PBS and stimulated with the high dose of LPS 7 days later. Animals were randomly assigned to the treatment groups; some received only PBS, but most of the animals were treated with LPS at one of these points. No animal received LPS twice. 8 WT and 7 KO received 50 μg/kg LPS; 6 WT and 5 KO were perfused at 8 h, 8 WT and 6 KO at 24 h after 2.5 mg/kg LPS; 14 WT and 7 KO were treated with PBS. 8 or 24 h after PBS- or high-dose-LPS-treatment the animals were killed by terminal anesthesia with pentobarbital (i.p.; approximately 100–160 mg/kg, Merial GmbH, Hallbergmoos, Germany) and transcardially perfused with ice-cold 0.9% saline. Blood samples were collected via cardiac puncture with a sterile heparinized syringe

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