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Systemic lipopolysaccharide (LPS)-induced microglial activation results in different temporal reduction of CD200 and CD200 receptor gene expression in the brain

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ABSTRACT

LPS activates microglia, which are normally maintained in a quiescent state by CD200–CD200 receptor (CD200R) interaction. MAC-1 (a microglia marker) mRNA expression was increased in mice brains up to 1 year post LPS administration (i.p.). Minocycline treatment did not prevent LPS (5 mg/kg)-induced increase in MAC-1 mRNA but reduced that induced by 0.1 mg/kg LPS. CD200R mRNA decreased starting at 4 h, whereas CD200 mRNA increased at 4 h and decreased at 1 year post LPS inoculation. Thus, LPS-induced changes in CD200–CD200R equilibrium might keep microglia chronically activated. Minocycline does not effectively inhibit microglia activation induced by high-dose LPS.

1. Introduction

Lipopolysaccharide (LPS), a bacterial endotoxin, activates microglia and can cause neurodegeneration (reviewed in Dutta et al., 2008; Perry and Andersson, 1992; Zielasek and Hartung, 1996). A single systemic injection of LPS can induce chronic activation of microglia accompanied by neuronal loss (Qin et al., 2007), or augment microglia activation in the central nervous system and accelerate the progression of a neurodegenerative disease (Cunningham et al., 2009).

Activated microglia contribute to neuroinflammation in the brain through the production of inflammatory molecules such as cytokines, nitric oxide, superoxide, eicosanoids, and quinolinic acid (Liu and Hong, 2003; Wojtera et al., 2005). Activated microglia have been found and implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (Dewil et al., 2007; Maccioni et al., 2009; McGeer et al., 1988). Various clinical trials have been carried out or are underway in order to evaluate the impact of inhibiting microglia on the progression of neurodegenerative diseases (Gordon et al., 2007; NINDS-NET-PD-Investigators, 2008; Zhang et al., 2008).

In the brain it has been suggested that neurons maintain microglia in a quiescent state through interactions between CD200 (OX-2) and its receptor (Neumann, 2001). CD200 is an integral membrane glycoprotein of the immunoglobulin superfamily (IgSF) with two IgSF domains: a transmembrane domain and a short cytoplasmic domain (Barclay and Ward, 1982; Clark et al., 1985) which lacks intracellular signaling domains. Its receptor is also a membrane glycoprotein with two IgSF domains, but with a larger cytoplasmic domain with signaling capacity (Wright et al., 2000). In the CNS, CD200 is expressed on endothelial cells and neurons whereas the CD200 receptor (CD200R) is expressed on cells of myeloid origin such as microglia and macrophages (Minas and Liversidge, 2006; Neumann, 2001). Several studies have demonstrated that disruption of the CD200/CD200R interaction results in activated microglia and a worsening of neuroinflammation (Deckert et al., 2006; Hoek et al., 2000; Meuth et al., 2008).

In this study the expression of mRNA of a marker of microglia activation (MAC-1), CD200 and CD200R has been analyzed after systemic administration of LPS using a dose which has been reported to produce chronic microglia activation and neuronal loss (Qin et al., 2007). The mRNA levels of MAC-1 were upregulated at 1 day and up to 1 year post LPS administration. On the other hand, CD200 mRNA levels were increased earlier than MAC-1, came down at 1 day and were decreased by 1 year, whereas the levels CD200R were downregulated from 4 h up to 1 year post LPS administration.

2. Materials and methods

2.1. Animals, LPS administration and minocycline treatment

In this study seventy C57BL/6 mice (8 to 12 weeks old) were used and were supplied by the breeding unit at the Health Science Center,

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Kuwait University, Kuwait. The mice were kept 4–8/cage with food and water *ad libitum*. Efforts were made to minimize animal numbers and suffering. All procedures were approved by the Kuwait University Health Sciences Centre animal care committee.

Mice received a single intraperitoneal dose of LPS from *Escherichia coli* (strain O111:B4, Sigma-Aldrich, St Louis, MO, USA, $n=44$) 5 mg/kg or its solvent (phosphate buffered saline, PBS, $n=26$). The dose of LPS was selected based on a study which reported that this dose produced neurodegeneration and chronic microglia activation, which could be observed at 10 months post administration (Qin et al., 2007). Another group of mice received a lower dose of LPS, 0.1 mg/kg. Mice were treated i.p. daily with minocycline (Sigma-Aldrich, St Louis, MO, USA) or its vehicle (PBS), commencing 2 days before LPS inoculation. Control mice were injected with 100 µl PBS daily, while the minocycline-treated LPS-inoculated mice received 50 mg/kg of the drug once daily for 3 days (for mice sacrificed at 4 h or 1 day post LPS inoculation) or a month (for mice sacrificed at 1 year post LPS inoculation). These doses were chosen based on those reported to reduce LPS-induced microglia activation (Henry et al., 2008). Animals were weighed and checked for signs of disease periodically. On the appropriate day post inoculation and treatment mice were deeply anaesthetized with halothane, sacrificed by decapitation, brains were dissected out at 4 h, 1 day and 1 year after LPS inoculation, snap frozen on dry ice and kept at −70 °C until mRNA extraction.

2.2. Real time RT-PCR

Gene transcripts of MAC-1, CD200 and CD200R were quantified in brains from minocycline-treated and PBS-treated LPS-inoculated and uninoculated mice by real time PCR. Total RNA was extracted from half of the fresh frozen brains and reverse-transcribed as described previously (Masocha et al., 2004). The real time PCR, was performed in triplicate 20 µl reactions containing 1× Platinum® SYBR® Green qPCR Supermix-UDG (Invitrogen), 125 nM of forward and reverse primers for cyclophilin and 500 nM for MAC-1, CD200 and CD200R primers, and 1 µl of cDNA on an ABI Prism® 7500 sequence detection system (Applied Biosystems). The primer sequences which were used are listed in Table 1. The primers were designed with the aid of Primer Express software version 3.0 (Applied Biosystems), compared to existing database at GenBank using BLAST to ensure specificity and ordered from Invitrogen. Ten-fold dilutions of a cDNA sample were amplified to control amplification efficiency for each primer pair. Thereafter, the Ct values for all cDNA samples were obtained. The amount of transcripts of individual animal sample ($n=4$ to 8 per group) was normalized to cyclophilin (ΔC_t). The relative amount of target gene transcripts was calculated using the $2^{-\Delta\Delta Ct}$ method as described previously (Livak and Schmittgen, 2001). These values were then used to calculate the mean and standard error of the relative expression of the target gene mRNA in the brain of LPS and solvent-treated mice.

Table 1
PCR primer sequences of cyclophilin, MAC-1 CD200 and CD200R.

Gene	Polarity	Sequence 5′to 3′	GenBank[a]
Cyclophilin	Sense anti-sense	GCTTTTCGCCGCTTGCT CTCGTCATCGGCCGTGAT	X52803
MAC-1	Sense anti-sense	TGCTTACCTGGGTTATGCTTCTG CCGAGGTGCTCCTAAAACCA	NM-008401
CD200	Sense anti-sense	CTGTGAGGGATTTGACTTTTTGC CCGAGGCACTCGACTTCCT	NM-010818
CD200R	Sense anti-sense	GGAAAACCAGAAAACCGAAATG CCCCCATATTAAGAGCACTGCTA	NM-021325

[a] GenBank accession numbers of sequences used for primer design.

2.3. Statistical analyses

Statistical analyses were performed using Mann Whitney *U* test, two-way ANOVA or one-way ANOVA followed by Newman–Keuls multiple comparison test. The differences were considered significant at $p<0.05$. The results in the text and figures are expressed as the means ± S.E.M.

3. Results

3.1. LPS-induced sickness behaviour

LPS-inoculated mice significantly lost weight (10.1 ± 1.1% loss) by 24 h post LPS administration compared to solvent-injected mice (Fig. 1A). However, the mice recovered and there were no subsequent weight differences between solvent and LPS-inoculated mice throughout the experiment up to 1 year post LPS administration (Fig. 1B).

3.2. Early expression of MAC-1, CD200 and CD200R transcripts in the brain after LPS administration

For the early time points post LPS administration, mRNA levels from brains of mice sacrificed at 4 h and 1 day after LPS administration

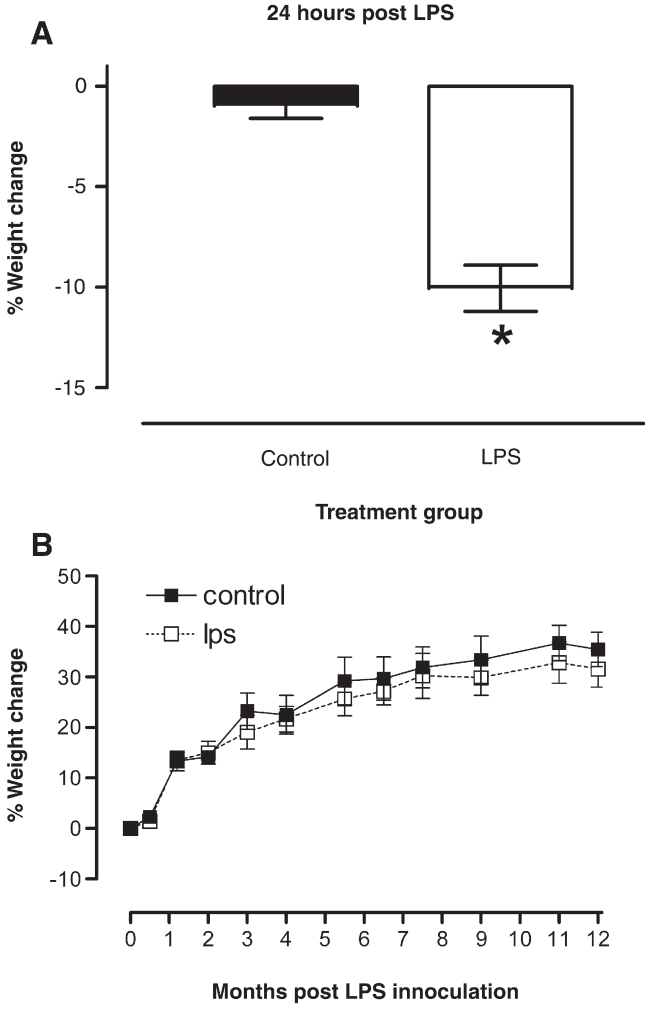

Fig. 1. Body weight changes in solvent (PBS)- or LPS-inoculated mice. Body weight is expressed as a percentage change of the pre-PBS or -LPS inoculation weight at day 0. Each point represents the mean ± S.E.M of the values obtained from 4 to 7 animals. Statistically significant differences in comparison with solvent-injected control animals: *$p<0.05$ (Mann Whitney *U* test). There were no significant differences between solvent and LPS-inoculated animals in figure B (two-way ANOVA).