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CD200 fusion protein decreases microglial activation in the hippocampus of aged rats

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ABSTRACT

The glycoprotein, CD200, is primarily expressed on neurons and its cognate receptor CD200R is expressed principally on cells of the myeloid lineage, including microglia. The interaction of CD200 with its receptor plays a significant role in maintaining microglia in a quiescent state and therefore a decrease in CD200 expression in brain is associated with evidence of microglial activation. Conversely, activation of CD200R, for example using a CD200 fusion protein (CD200Fc), should result in a decrease in microglial activation. Here we assessed the effect of delivery of CD200Fc intrahippocampally on microglial activation and on long-term potentiation (LTP) in perforant path-granule cell synapses in young and aged rats. We hypothesized that the age-related changes in microglial activation would be attenuated by CD200Fc resulting in an improved ability of aged rats to sustain LTP. The data indicate that expression of markers of microglial activation including major histocompatibility complex Class II (MHCII) and CD40 mRNA, as well as MHCII immunoreactivity, were increased in hippocampus of aged, compared with young, rats and that these changes were associated with a deficit in LTP; these changes were attenuated in hippocampal tissue prepared from aged rats which received CD200Fc. Microglial activation and a deficit in LTP have also been reported in lipopolysaccharide (LPS)-treated rats and, here, we report that these changes were also attenuated in CD200Fc-treated animals. Thus the negative impact of microglial activation on the ability of aged and LPS-treated rats to sustain LTP is ameliorated when CD200R is activated by CD200Fc.

1. Introduction

CD200 is a type-1 membrane glycoprotein which has been identified as an immuno-suppressive molecule. It is expressed on several cell types and, in the brain, CD200 is expressed on neurons (Barclay et al., 2002) and oligodendrocytes (Koning et al., 2009) but not on microglia (Lyons et al., 2007a). CD200 was reported to be expressed on reactive astrocytes in lesions from postmortem multiple sclerosis brains (Koning et al., 2009) but recent evidence from this laboratory suggests that it is also expressed on astrocytes prepared from 1 day-old mice (Costello et al., 2011). The receptor for CD200, CD200 receptor (CD200R), is also a membrane glycoprotein and has an NPXY signaling motif containing three tyrosine residues in its intracellular domain (Snelgrove et al., 2008; Wright et al., 2000). This contrasts with CD200, which has a short cytoplasmic domain with no signaling motifs (Barclay et al., 2002). CD200R expression is restricted primarily to cells of the myeloid lineage and therefore, in the brain, has been identified on microglia (Barclay et al., 2002; Koning et al., 2009) but not on neurons (Lyons et al., 2007a) or astrocytes.

The complementary expression of ligand and receptor on neurons and microglia respectively, suggested that the interaction between CD200 and its receptor may play a role in modulating microglial activation and recent evidence supports this contention. Thus the lipopolysaccharide (LPS)- and amyloid-β (Aβ)-induced increase in expression of cellular markers of microglial activation was inhibited when glia were co-cultured with neurons and this effect of neurons was attributed to CD200–CD200R interaction since it was blocked by an anti-CD200 antibody (Lyons et al., 2007a; Lyons et al., 2009). This finding, and others, suggests that interaction of CD200 with its receptor modulates microglial activation. This has been confirmed by analysis in CD200-deficient mice; thus microglial and/or macrophage activation occurs to a greater extent in these mice compared with wildtype mice in several models of inflammation, for example facial nerve transection, experimental autoimmune encephalomyelitis (EAE), an animal model of arthritis (Hoek et al., 2000) and experimental autoimmune uveoretinitis (Broderick et al., 2002). Consistently, the decrease in EAE-like symptoms in *Wld*^{s} mice has been attributed to increased expression of CD200 on spinal cord neurons (Chitnis et al., 2007). Conversely, administration of a CD200 fusion protein, containing the ectodomain of CD200 bound to a murine IgG2a module, ameliorates the inflammatory changes observed in collagen-induced arthritis (Gorczynski et al., 2001, 2002).

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One consequence of the neuroinflammatory changes which accompany microglial activation is a deficit in synaptic plasticity, specifically long-term potentiation (LTP) (Lynch et al., 2007; Nolan et al., 2005). Here we considered that if the age-related microglial activation was reduced by activating CD200R, then the ability of rats to sustain LTP may be improved and therefore we set out to investigate the effect of a CD200 fusion protein (CD200Fc) on microglial activation and LTP in aged rats. We argued that activation of CD200R by CD200Fc would also attenuate the LPS-induced microglial activation and consequently reduce the LPS-induced deficit in LTP. The data show that intrahippocampal delivery of CD200Fc ameliorated the age-related and LPS-induced activation of microglia and the accompanying deficit in LTP suggesting that CD200R activation, by modulating microglial activation, positively impacts on neuronal function.

2. Materials and methods

2.1. Animals

Young (3 months; 250–350 g) and aged (20–22 months; 550–600 g) male Wistar rats (Bantham and Kingman, UK) were housed in a controlled environment (temperature: 20–22 °C; 12:12 h light/dark cycle) in the BioResources Unit, Trinity College, Dublin. Animals had free access to food and water and were maintained under veterinary supervision for the duration of the experiment. All experiments were carried out under license from the Department of Health and Children (Ireland) and with ethical approval from the Trinity College Ethical Committee.

2.2. Analysis of LTP in vivo

Rats were anaesthetized by intraperitoneal injection of urethane (1.5 g/kg) and the absence of a pedal reflex was considered to be an indicator of deep anesthesia. In the first series of experiments, young and aged animals were subdivided into an experimental and a control group, with six animals per group, and all animals received a single unilateral injection. Animals were placed in a stereotaxic frame, the skull was exposed and a dental drill was used to make a small hole to allow the intrahippocampal injections to be made. The experimental group received CD200Fc intrahippocampally (2 μg/μl; 5 μl injection volume; 0.8 mm lateral and 3.5 mm dorsoventral to Bregma) and the control groups received sterile saline (5 μl). The recombinant mouse CD200Fc used here (murine myeloma cell line, NSO-derived; Cat. No. 3355-CD; R&D Systems, US) was prepared by fusing the N-terminal domain of CD200 (Gln31-Gly232) to human IgG_1); it is known to bind CD200R1 in a linear manner within the range 0.4–25 ng/ml but its affinity for other CD200R family members is not known. Preliminary experiments were undertaken to assess the effect, if any, of the Ig tag on the CD200Fc construct and no differences between the ability of saline-injected and Ig-injected rats on LTP were identified. Therefore saline was used as the control in all further experiments. Following injection, bore holes were made in the skull to enable placement of the electrodes; a bipolar stimulating electrode was stereotaxically positioned in the perforant path (4.4 mm lateral to Bregma) and a unipolar recording electrode was placed in the dorsal cell body region of the dentate gyrus (2.5 mm lateral and 3.9 mm posterior to Bregma). Following a period of stabilisation, test shocks were delivered at 30 s intervals and stable baseline responses were recorded for 10 min prior to tetanic stimulation which was delivered 1 h after intrahippocampal injection. The tetanus consisted of three trains of high-frequency stimuli (250 Hz for 200 ms; 30 s inter-train interval) delivered to the perforant path and following this stimulation, recording at test shock frequency resumed for the remainder of the experiment (Martin et al., 2002). Animals were killed by cervical dislocation and this was 2 h after intrahippocampal injection. The slope of the excitatory post-synaptic potential (EPSP) was used as a measure of excitatory synaptic transmission in the dentate gyrus.

In the second series of experiments, young animals were divided into four groups of six rats: control rats, rats which received an intraperitoneal injection of LPS (100 μg/kg; from *Escherichia coli*, serotype EH100(Ra), TLR grade; Alexis Biochemicals, UK; Cat. No. ALX-581-010-L002), rats which received CD200Fc intrahippocampally (2 μg/μl; 5 μl injection volume) and rats which received both LPS and CD200Fc. All rats were placed in the stereotaxic frame, as described above, for administration of a single unilateral injection of either saline or CD200Fc, and were then removed from the frame. Ten minutes later, rats received a single intraperitoneal injection of either saline or LPS. Three hours later, rats were replaced in the stereotaxic frame to enable placement of the electrodes and analysis of LTP, as described above. In this case, recording started 4 h after LPS injection (with the tetanus delivered 10 min later) and animals were killed approximately 5 h after LPS.

At the end of the period of recording, rats were killed by cervical dislocation. The lateral third of the injected side of the brain was coated with OCT compound (Sakura Tissue-Tek, Netherlands), immersed in isopentane at −30 °C and stored at −80 °C until sections were prepared. Cryostat sections (10 μm) were mounted on Superfrost® Plus slides (Thermo Scientific, Germany), air-dried for 30 min and stored at −20 °C until used for immunohistochemical analysis of MHCII. The remaining tissue (medial hippocampus from the injected side) was snap-frozen and used to prepare mRNA for PCR analysis.

2.3. Real-time PCR analysis of cytokines and cell surface markers

Total RNA was extracted from snap-frozen hippocampal tissue using a NucleoSpin® RNAII isolation kit (Macherey–Nagel Inc., Germany) according to the manufacturer's instructions. RNA integrity and total RNA concentration were assessed, and cDNA synthesis was performed as described previously (Lyons et al., 2011). Real-time PCR was performed using Taqman Gene Expression Assays (Applied Biosystems, Germany) which contain forward and reverse primers, and a FAM-labeled MGB Taqman probe for each gene of interest. The assay IDs for the genes examined in this study were as follows: MHCII (Rn01768597_m1), CD40 (Mm00441895_m1), CD11b (Mm001271265_m1), CD68 (Rn01495631_g1), inducible nitric oxide synthase (iNOS) (Rn00561646_m1), interferon gamma-induced protein 10 (IP-10; Rn00594648_m1) and monocyte chemotactic protein-1 (MCP-1; Rn00580555_m1). All real-time PCR was conducted using an ABI Prism 7300 instrument (Applied Biosystems, Germany). A 20 μl volume was added to each well (9 μl of diluted cDNA, 1 μl of primer and 10 μl of Taqman® Universal PCR Master Mix). Samples were assayed in duplicate in one run (40 cycles), which consisted of three stages, 95 °C for 10 min, 95 °C for 15 s for each cycle (denaturation) and finally the transcription step at 60 °C for 1 min. β-actin was used as the endogenous control to normalize gene expression data, and β-actin expression was conducted using a gene expression assay containing forward and reverse primers (primer limited) and a VIC-labeled MGB Taqman probe from Applied Biosystems (Germany; Assay ID: 4352341E). Gene expression was calculated relative to the endogenous control samples and to the control sample giving an RQ value (2^{-DDCt}, where Ct is the threshold cycle).

2.4. Staining of MHCII

Frozen cryostat sections (10 μm) were prepared as described previously (Nolan et al., 2005). Sections were fixed in ice-cold