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Classical activation of microglia in CD200-deficient mice is a consequence of blood brain barrier permeability and infiltration of peripheral cells

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ABSTRACT

The interaction between CD200, expressed on several cell types, and its receptor CD200R, expressed on cells of the myeloid lineage, has been shown to be an important factor in modulating inflammation in macrophage function in several conditions including colitis and arthritis. More recently its modulatory effect on microglial activation has been identified and CD200-deficiency has been associated with increased microglial activation accompanied by increased production of inflammatory cytokines. The response of glia prepared from CD200-deficient mice to stimuli like lipopolysaccharide (LPS) is markedly greater than the response of cells prepared from wildtype mice and, consistent with this, is the recent observation that expression of Toll-like receptor (TLR)4 and signalling through NFκB are increased in microglia prepared from CD200-deficient mice. Here we show that glia from CD200-deficient mice are also more responsive to interferon-γ (IFNγ) which triggers classical activation of microglia. We investigated the effects of CD200-deficiency *in vivo* and report that there is an increase in expression of several markers of microglial activation including tumor necrosis factor (TNF)-α, which is a hallmark of classically-activated microglia. These changes are accompanied by increased IFNγ, and the evidence suggests that this is produced by infiltrating cells including T cells and macrophages. We propose that these cells enter the brain as a consequence of increased blood brain barrier (BBB) permeability in CD200-deficient mice and that infiltration is assisted by increased expression of the chemokines, monocyte chemotactic protein-1 (MCP-1), IFNγ-induced protein-10 (IP-10) and RANTES. This may have implications in neurodegenerative diseases where BBB permeability is compromised.

1. Introduction

Microglia, like macrophages, are plastic cells, responding to different stimuli by adopting different morphological and functional phenotypes indicative of their broad array of functions (Lynch, 2009). These cells are the immune cells of the brain and therefore respond to pathogen-associated, and danger-associated, molecular patterns which trigger the responses that result from insult. An acute stimulus generally induces release of inflammatory mediators, but this relatively brief inflammatory state is followed by restoration of microglia to their resting state. In contrast, exposure of cells to chronic activation is undesirable and can result in damage to neighboring cells.

A number of endogenous mechanisms ensure that microglial activation is kept in check and these include the interaction with other cells through ligand–receptor binding. Among the ligand–receptor pairs is CD200–CD200R which has complementary cell expression patterns characteristic of this method of controlling microglial activation. Thus CD200 is expressed on several cell types including neurons (Lyons et al., 2007a; Webb and Barclay, 1984) and astrocytes (Costello et al., 2011) whereas CD200R is expressed primarily on cells of the myeloid lineage and on microglia in the brain (Barclay et al., 2002; Koning et al., 2009). The evidence has indicated that decreased expression of CD200, which occurs with age (Cox et al., 2012; Frank et al., 2006), Aβ treatment (Lyons et al., 2007a) or in the brain of AD patients (Walker et al., 2009), is associated with the type of microglial activation that is characterized by inflammatory changes (Colton and Wilcock, 2010). At least in some cases, these inflammatory changes are attenuated by a CD200 fusion protein (CD200Fc) which stimulates CD200R activation (Cox et al., 2012; Lyons et al., 2012), although the evidence indicates that the reversal is incomplete.

We have recently shown that glial cells prepared from $CD200^{-/-}$ mice respond more profoundly to Toll-like receptor (TLR) agonists, specifically lipopolysaccharide (LPS) which interacts with TLR4, and Pam_3CSK_4 which interacts with TLR2 (Costello et al., 2011);

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expression of both receptors was increased on microglia prepared from CD200$^{-/-}$ mice, compared with wildtype mice, and this was associated with a genotype-related increase in signaling through NFκB. Thus the loss of CD200 in CD200-deficient mice is accompanied by a number of changes that play a role in modulating microglial activation.

In the present study, we set out to evaluate the changes that accompany the loss of CD200 *in vivo* with the objective of increasing our understanding of the mechanisms that modulate microglial function. First, we showed that interferon (IFN)-γ, which induces a classically-activated microglial phenotype exerted a greater effect in cultured cells from CD200$^{-/-}$, compared with wildtype, mice. *In vivo*, CD200 deficiency was associated with increased expression of markers of microglial activation including tumor necrosis factor (TNF)-α, which is a hallmark of classically-activated microglia, in the absence of any stimulus. Increased expression of IFNγ was observed in hippocampus of CD200$^{-/-}$ mice, although it is generally considered that IFNγ is not produced by resident cells in the brain (Lynch, 2010; Renno et al., 1995). The probable explanation for this is that there was a significant infiltration of T cells and macrophages into the brain of CD200$^{-/-}$ mice, and the data suggest that cell infiltration is a consequence of the increase in blood brain barrier (BBB) permeability and increased expression of the chemokines, monocyte chemotactic protein-1 (MCP-1), IFNγ-induced protein-10 (IP-10) and RANTES (also known as CCL5).

The data highlight the consequences of infiltration of peripheral immune cells on microglial function and suggest that the inflammatory changes which occur in neurodegenerative diseases might be modulated by targeting the BBB.

2. Methods

2.1. Animals

C57BL/6 mice (wildtype) and CD200-deficient mice were used in this study. Mice were bred in an SPF housing facility in the Bio-Resources Unit, Trinity College, Dublin and were maintained under veterinary supervision in a controlled environment (12-hour light-dark cycle; 22–23 °C) for the duration of the experiment. In one series of experiments, wildtype mice were anaesthetized with urethane (1.5 g/kg; Sigma Aldrich; IRE) and injected with IFNγ (5 μl @ 1 μl/min; 50 ng/ml; R&D Systems, UK; co-ordinates: 0.34 mm caudal and 1 mm lateral to bregma, at a depth of 2.1 mm. After 3 h, animals were perfused intracardially with ice-cold PBS (20 ml). In all experiments, the brain was rapidly removed and placed on ice, the cerebellum, brain stem, olfactory lobes and hippocampus were removed and the remaining tissue was taken to prepare a single cell suspension for flow cytometric analysis as described below. Animal experimentation was performed under a licence granted by the Minister for Health and Children (Ireland) under the Cruelty to Animals Act 1876 and the European Community Directive, 86/609/EEC, and in accordance with local ethical guidelines.

2.2. MRI analysis to assess BBB permeability and blood flow

MRI analysis, to assess extravasation of the gadolinium-based contrast agent, gadopentate dimeglumine (Magnevist; Clissmann, Ireland) as an indicator of BBB permeability, was carried out on a small rodent Bruker Biospec (Bruker Biospin, Germany) system with a 7 Tesla magnet and a 30 cm core. Anaesthesia was induced and maintained using isoflurane (5% in 100% O_2 and 1.5–2% at 1 l/min of 100% O_2, respectively). Respiration rate was monitored and body temperature was controlled throughout.

Mice were placed into an MR-compatible cradle and positioned in a stereotaxic frame. The right lateral tail vein was cannulated using a 30 gauge in-dwelling, paediatric intravenous cannula needle (Introcan, Ireland) to which an 80 cm long polyethylene tubing cannula extension, connected to a 3-way tap, was attached; this allowed delivery of contrast agent (200 μl/mouse) without disturbing the animals and enabled pre- and post-contrast measurements to be determined within the same contrast scan. Contrast imaging was carried out using a 10 repetition T_1-weighted fast low angle shot (FLASH) sequence; the repetition duration was 2 min, 11 s and the 15-slice protocol had dimensions of 128 × 128 voxels per slice. Pre-contrast measurements were acquired on the first repetition of the scan to provide a contrast-free baseline after which the contrast agent was injected; the following 9 scan repetitions recorded the passage of the bolus throughout the brain. Gadolinium-enhanced contrast images acquired from the BBB permeability studies were analysed in the MIPAV software package. The average signal intensity changes in each treatment group were plotted against repetition. Anatomically-distinct ROIs, viewed in the first repetition, were overlaid with a 2 × 2 voxel square and the average pre-contrast intensity was measured. The ROI squares were copied into the same locations in the remaining 9 repetitions for each mouse brain, measuring the contrast change following injection of the gadolinium-based contrast agent at the commencement of the second repetition. Values for each data set were normalised to the pre-contrast measurement and expressed as a proportion of the pre-contrast value.

Quantitative blood flow and volume measurements were investigated using a bolus-ASL methodology (Kelly et al., 2010). This method uses a bolus-tracking ASL sequence to provide groups of 11 one slice images on a single time curve following the passage of a 3 s bolus through the imaging slice. Control and labelled images were created for wildtype and CD200-deficient mice and corresponding pairs were subtracted to provide perfusion-weighted maps. Mean values for cortical and hippocampal ROIs, manually drawn on perfusion-weighted images, were plotted against the acquisition time point in the bolus tracking ASL sequence. The curve was fitted to the solution of the non-compartmental cerebral perfusion ASL model (Kelly et al., 2009), and the curve-fitting utility in Mathematica was used to find the least squares fit to the experimental curves. The mean transit time (MTT) and capillary transit time (CTT) were calculated from the first and second statistical moments of the signal-time curves, respectively.

Following MRI scanning, animals were anaesthetised with urethane (1.5 g/kg; Sigma Aldrich; IRE) and perfused intracardially with ice-cold PBS (20 ml). The brain was rapidly removed and placed on ice, the cerebellum and olfactory bulbs were removed and the brain was bisected along the midline. The hippocampus was dissected free and snap-frozen in liquid nitrogen, for later analysis by PCR and/or ELISA, and the remaining brain tissue was used to prepare a single cell suspension for analysis by flow cytometry.

2.3. Preparation of cultured glia

Mixed glial cells were prepared from 1 day-old wildtype and CD200$^{-/-}$ mice (BioResources Unit, Trinity College, Dublin, Ireland) as described (Costello et al., 2011). To prepare mixed glia, whole brain minus cerebellum was roughly chopped, added to pre-warmed Dulbecco's modified Eagle medium (DMEM; Gibco BRL, Ireland) containing 10% foetal bovine serum, penicillin (100 U/ml) and streptomycin (100 U/ml), triturated and filtered through a sterile mesh filter (70 μm). Samples were centrifuged (2000*g*, 3 min, 20 °C), the pellet was resuspended in warmed DMEM, cells were seeded in 6 ml flasks (1×10^6 cells/flask) and incubated for 2 h before addition of warmed DMEM. Cells were grown for 10–12 days at 37 °C in a humidified 5% CO_2:95% air environment and