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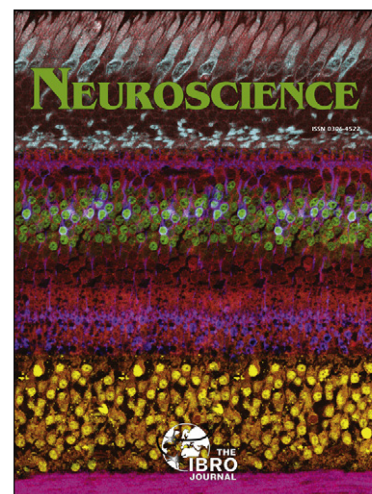

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The neural cell adhesion molecule-derived peptide, FGL, attenuates lipopolysaccharide-induced changes in glia in a CD200-dependent manner

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Running title **FGL action is CD200-dependent**

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ABSTRACT

Fibroblast growth loop (FGL) is a neural cell adhesion molecule (NCAM)-mimetic peptide that mimics the interaction of NCAM with fibroblast growth factor receptor (FGFR). FGL increases neurite outgrowth and promotes neuronal survival *in vitro*, and it has also been shown to have neuroprotective effects *in vivo*. More recent evidence has indicated that FGL has anti-inflammatory effects, decreasing age-related changes in microglial activation and production of inflammatory cytokines. These changes have been associated with an FGL-induced increase in expression of the glycoprotein, CD200, which interacts with its receptor to help maintain microglia in a quiescent state. However whether the FGL-induced anti-inflammatory effects are CD200-dependent has not been examined. The objective of this study was to address this question. Mixed glia were prepared from brain tissue of neonatal wildtype and CD200-deficient mice and preincubated with FGL prior to stimulation with lipopolysaccharide (LPS). Cells were assessed for mRNA expression of markers of microglial activation, CD11b, CD40 and intercellular adhesion molecule 1 (ICAM) and also the inflammatory cytokines, interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α, while supernatant concentrations of these cytokine were also assessed. LPS significantly increased all these parameters and the effect was greater in cells prepared from CD200-deficient mice. Whereas FGL attenuated the LPS-induced changes in cells from wildtype mice, it did not do so in cells from CD200-deficient mice. We conclude that the FGL-induced changes in microglial activation are CD200-dependent and demonstrate that the interaction of astrocytes with microglia is critically important for modulating microglial activation.

KEYWORDS: Microglial activation, CD200, Fibroblast growth loop (FGL), inflammatory cytokines.

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HIGHLIGHTS

- LPS increased microglial activation and production of inflammatory cytokines in cultured cells
- The LPS-induced changes were greater in cells from $CD200^{-/-}$, compared with wildtype, mice
- FGL attenuated the LPS-induced changes in cells from wildtype, but not $CD200^{-/-}$, mice
- Astrocytes, which express CD200, play an important role in modulating microglial activation

INTRODUCTION

Neural cell adhesion molecule (NCAM), a glycoprotein which is widely expressed in the brain, comprises an extracellular region with 5 N terminal Ig and 2 fibronectin-type III (F3 modules). NCAM interacts with growth factor receptors including fibroblast growth factor receptor (FGFR) and the FGFR-1 binding site of NCAM is now known to be a small peptide loop in the F3 region (Kiselyov et al., 2003). The 15 amino acid peptide, FGL peptide, which encompasses the FGFR-1 binding site of NCAM, exerts a plethora of effects. It enhances hippocampal function (Cambon et al., 2004, Dallerac et al., 2011) and plays a role in neuronal development (Cambon et al., 2004, Li et al., 2009, Dallerac et al., 2011). FGL has also been shown to be protective against 6-hydroxydopamine and amyloid-β (Aβ) *in vitro* (Neiiendam et al., 2004) and decreases neuronal damage in hippocampal organotypic slice cultures subjected to oxygen-glucose (Skibo et al., 2005). Protective effects of FGL *in vivo* have also been reported; thus it has been shown to attenuate the increased Aβ-immunoreactivity and deficit in cognitive function induced by intracerebroventricular administration of pre-aggregated $A\beta_{25-35}$ (Klementiev et al., 2007).

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More recently, an anti-inflammatory effect of FGL has been observed; the evidence indicated that FGL restored the age-related imbalance in hippocampal concentrations of IGF-1 and IFNγ and consequently attenuated the glial cell activation associated with aging (Downer et al., 2009, Ojo et al., 2011). Administration of FGL to aged rats for 3 weeks was shown to attenuate the age-related deficit in LTP and the increase in microglial activation (Downer et al., 2010); this was coupled with an FGL-induced increase in CD200 on neurons (Downer et al., 2010), particularly boutons (Ojo et al., 2012), and also on astrocytes (Ojo et al., 2012).

CD200, which is expressed on neurons and endothelial cells, by interacting with its cognate receptor, CD200R which is expressed on microglia but not other cells in the brain, plays an important role in maintaining the resting non-inflammatory state. Thus microglia of CD200-deficient mice spontaneously exhibit many features of activation, including a less ramified morphology and increased expression of markers like CD11b and CD45 and increased inflammatory changes associated with experimentally-induced autoimmune diseases like experimental autoimmune encephalomyelitis and uveitis (Hoek et al., 2000, Copland et al., 2007). Recent data have indicated that glia prepared from CD200-deficient mice responded more profoundly to lipopolysaccharide (LPS) than cells from wildtype mice (Costello et al., 2011); these cells exhibited increased expression of markers of microglial activation and increased production of inflammatory cytokines, interleukin (IL)-1β, IL-6 and tumour necrosis factor-α (TNFα).

Our previous findings indicated that the FGL-induced decrease in microglial activation was associated with increased CD200 expression; in this study, we set out to evaluate whether the effects of FGL were CD200-dependent. We demonstrate that pretreatment of cultured glia with FGL attenuated the LPS-induced increase in markers of microglial activation and proinflammatory production in cells prepared from wildtype mice, but exerted

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no effect on the LPS-induced changes in CD200-/- mice. The data indicate that the ability of FGL to modulate microglial activation is dependent on CD200.

EXPERIMENTAL PROCEDURES

Preparation of cultured cortical rat mixed glia

Mixed glial cells, isolated astrocytes and isolated microglia were prepared from neonatal wildtype or CD200$^{-/-}$ mice as described previously (Costello et al., 2011). In some experiments, mixed glia were pretreated with FGL (10µg/ml) for 24 hours before being incubated in the presence or absence of LPS (1µg/ml) for a further 24 hours. This regime was chosen following preliminary experiments which showed that pretreatment induced significantly greater effects than co-treatment at 10µg/ml FGL. We examined the effects of LPS after 24 hours since, in a previous study, we observed that the changes in cytokines were maximal at this time (Minogue et al., 2012). In some experiments, purified astrocytes were incubated in the presence or absence of FGL (10µg/ml) for 24 hours to assess its effect on CD200 protein expression.

In a separate series of experiments, cell membranes were isolated from cultured astrocytes prepared from C57BL/6 mice using a subcellular protein fractionation kit (Thermo Scientific, US). Confluent astrocytes were incubated in trypsin-EDTA (1ml, 15 min, 37ºC), the cell suspension was centrifuged (500 x g, 5 min), the pellet was washed and cells were resuspended in phosphate-buffered saline (PBS, 1ml). Samples were centrifuged (500 x g, 3 min), the pellet was re-suspended in ice-cold Cytoplasmic Extraction Buffer (100µl, Thermo Scientific, US), incubated (4ºC, 10 min) and centrifuged (3,000 x g, 5 min). The pellet was resuspended in ice-cold Membrane Extraction Buffer (100µl, Thermo Scientific, US), incubated (4ºC, for 10 min) and centrifuged (3,000 x g, 5 min) to provide a supernatant which contained the membrane extract. Purified microglia were incubated in the presence or

absence of this membrane extract (20ng/ml) for 2 hours prior to the addition of LPS (1µg/ml) and incubation continued for 24 hours.

Quantitative real time PCR

RNA was isolated from cultures using the Nucleospin® RNAII KIT (Macherey-Nagel, Duren, Germany) and cDNA was prepared using High-Capacity cDNA RT kit according to the manufacturer's instructions (Applied Biosystems, UK). Real-time PCR for the detection of CD40, ICAM-1, CD11b, IL-1β, IL-6 and TNFα mRNA was performed with predesigned Taqman gene expression assays (Applied Biosystems, UK). The assay IDs were as follows: CD40 Mm00441891_m1, ICAM-1 Mm00516027_g1, CD11b Mm01271265_m1, IL-1β Mm00434228_m1, IL-6 Mm00446190_m1 and TNFα Mm00443258_m1. Samples were assayed on an Applied Biosystems 7500 Fast Real-Time PCR machine and gene expression was calculated relative to the endogenous control samples (β-actin) to give a relative quantification (RQ) value (2^{-DDCT}, where CT is threshold cycle).

Analysis of cytokine expression by ELISA

The concentrations of IL-1β and IL-6 were assessed by ELISA in supernatant from primary rat mixed glial cultures as previously described (Minogue et al., 2012). Briefly, 96-well plates (Nunc-Immuno plate with Maxisorp surface, Denmark) were coated with capture antibody (rat anti-mouse IL-1β (4µg/ml in PBS; R & D Systems, US), rat anti-mouse IL-6 (2µg/ml in PBS; BD Biosciences, UK), or goat anti-mouse TNFα antibody (0.8 µg/ml in PBS BD Biosciences, UK)) and incubated (overnight, 4ºC). Triplicate samples or standards (50µl) were added and plates were incubated (2 hours, room temperature) and washed before addition of detection antibody (600ng/ml in 2% goat serum for IL-1β or 200ng/ml in PBS containing 10% FBS for IL-6 and TNFα). Samples were incubated (2 hours, room

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temperature), washed, incubated with streptavidin-horseradish peroxidase conjugate (100µl; 1:200; 20 min, room temperature) and washed before addition of substrate solution (100µl; 1:1 H_2O_2:tetramethylbenzidine; R&D Systems, US). After colour development, the reaction was stopped by adding 50µl 1M H_2SO_4 and plates were read at 450nm (Labsystem Multiskan RC, UK).

Statistical Analysis

Data are expressed as means ± standard error of mean (SEM). Analysis of variance (ANOVA) was performed to determine whether significant differences existed between conditions. If this indicated significance ($p<0.05$), the appropriate a post-hoc test was used to determine which conditions significantly differ from each other. Two-tailed unpaired Students *t*-tests were also performed, where indicated, to compare treatment groups; significance was set as $p<0.05$.

RESULTS

LPS induced a significant increase in mRNA expression of CD40, ICAM-1 and CD11b in mixed glia prepared from wildtype and $CD200^{-/-}$ mice ($^{**}p<0.01$; $^{***}p<0.001$; ANOVA; A, $F_{(7,32)}=7.91$; B, $F_{(7,36)}=31.2$ and C, $F_{(7,34)}=60.95$; Figure 1). The LPS-induced increase was significantly greater in mixed glia prepared from $CD200^{-/-}$, compared with wildtype, mice ($^{\S}p<0.05$; $^{\S\S}p<0.01$; ANOVA). Pre-treatment with FGL attenuated these LPS-induced changes in mixed glia prepared from wildtype, but not $CD200^{-/-}$, mice ($^{++}p<0.01$; $^{+++}p<0.001$; ANOVA).

LPS stimulated a significant increase in mRNA expression and supernatant concentration of IL-1β in mixed glia prepared from wildtype mice ($^{***}p<0.001$; ANOVA; A, $F_{(7,25)}=83.24$ and B, $F_{(7,35)}=7.77$; Figure 2) and this effect was significantly greater in cells from $CD200^{-/-}$

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mice ($^{§§§}p<0.001$; ANOVA; wildtype vs CD200$^{-/-}$ mice). FGL significantly attenuated the LPS-induced change in IL-1β mRNA and supernatant concentration of IL-1β in glia prepared LPS from wildtype mice ($^{+++}p<0.001$; ANOVA) but not in cells prepared from CD200$^{-/-}$ mice.

LPS also significantly increased TNF-α mRNA and IL-6 mRNA, and supernatant concentrations of both cytokines in mixed glia from wildtype mice ($^{**}p<0.01$; $^{***}p<0.001$; ANOVA; A, $F_{(7,34)}=54.05$ and B, $F_{(3,12)}=73$; Figure 3, and A, $F_{(7,27)}=30.51$ and B, $F_{(7,34)}=1877$; Figure 4); this effect was further enhanced in cells from CD200$^{-/-}$ mice ($^{§§§}p<0.001$; ANOVA; wildtype vs CD200$^{-/-}$ mice; Figures 3,4). Pre-treatment with FGL attenuated the LPS-induced increase in mRNA expression and supernatant concentration of TNFα and IL-6 in mixed glia prepared from wildtype mice but not cells from CD200$^{-/-}$ mice ($^{++}p<0.01$, $^{+++}p<0.001$; ANOVA; Figures 3,4).

These data obtained in mixed glia indicate that the loss of CD200 markedly affects expression of markers of microglial activation; this is probably because astrocytes, which express CD200 (Costello et al., 2011), modulate LPS-induced changes in microglia. To confirm the presence of CD200 on astrocytes, we show that CD200 is expressed on CD11b^{-} cells in a mixed glial cell preparation and that expression was not detectable in cells prepared from CD200$^{-/-}$ mice (Figure 4A). The data also show that expression of CD200 on purified astrocytes was significantly upregulated in astrocytes exposed to FGL ($^{*}p<0.05$; Student's *t*-test for independent means; Figure 4B) which concurs with the evidence that FGL also increased expression on neurons (Downer et al., 2009).

Having confirmed CD200 expression on astrocytes we prepared purified microglia and pre-incubated them in the presence or absence of astrocytic membranes for 2 hours prior to the addition of LPS. LPS significantly increased IL-1β mRNA in microglia ($^{***}p<0.001$; ANOVA; $F_{(4,17)}=16.01$; Figure 6A) but pre-incubation with the membrane preparation

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significantly attenuated the LPS-induced change ($^{+++}p<0.001$; ANOVA; LPS alone versus LPS+astrocytic membrane). IL-1β was below the detectable levels in the supernatant obtained from these cells. LPS also increased mRNA expression and supernatant concentration of TNF-α and IL-6 ($^{***}p<0.001$; ANOVA; B, $F_{(7,35)}=7.77$ and C, $F_{(3,14)}=18.27$, and D, $F_{(3,21)}=5.63$ and E, $F_{(3,17)}=6.68$ respectively; Figure 6) and these changes were attenuated when cells were pre-incubated with the astrocytic membrane preparation ($^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$; ANOVA; Figure 6; LPS alone versus LPS+astrocytic membrane).

DISCUSSION

The central finding in this study is that FGL exerts its modulatory effects on LPS-induced activation in a CD200-dependent manner and that its expression on astrocytes is pivotal to this action.

We set out to compare the effects of LPS on mixed glial cultures prepared from $CD200^{-/-}$, compared with glia from wildtype, mice and, specifically, to assess whether the predicted FGL-associated modulatory effect was CD200-dependent. The data indicate that LPS increased mRNA expression of markers of microglial activation and inflammatory cytokine production in glia prepared from wildtype mice, but that this effect was significantly greater in glia prepared from $CD200^{-/-}$ mice. Importantly, whereas pre-treatment of glia with FGL significantly attenuated the LPS-induced changes in cells from wildtype mice, it exerted no effect on cells from $CD200^{-/-}$ mice indicating that its effect was CD200-dependent. Moreover, when LPS-treated microglia were incubated in the presence of a membrane preparation obtained from astrocytes, the induced increases in inflammatory cytokine production were completely blocked. This indicates that astrocytes play an important role in modulating microglial activation.

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LPS is a potent activator of microglia and previous work from this laboratory has demonstrated its ability to stimulate transcription and release of inflammatory mediators in mixed glial cultures (Lyons et al., 2009, Cowley et al., 2010, Watson et al., 2010, Costello et al., 2011). The present data reiterate these effects showing LPS-induced increases in mRNA expression of CD40, ICAM-1 and CD11b, as well as mRNA expression and release of IL-1β, TNFα and IL-6. Pre-incubation of cells in the presence of FGL completely blocked these LPS-induced changes. The ability of FGL to inhibit LPS-induced IL-1β production in mixed glia, and also microglia, has been reported and the earlier evidence indicated that this was IL-4-dependent (Downer et al., 2010). FGL also attenuates the IFNγ-induced increase in microglial activation, specifically expression of MHCII and CD40 mRNA; in this case the evidence suggested that FGL acted in an IGF-1-dependent manner (Downer et al., 2009). Significantly, IL-4 and IGF-1 both increased expression of CD200, suggesting that the effect of FGL was linked with increased CD200.

Here, we demonstrate that LPS induced a significantly greater effect on all parameters in glia prepared from $CD200^{-/-}$, compared with wildtype, mice. The LPS-induced increase in cytokine release from cells has previously been shown to be enhanced in the absence of CD200 (Costello et al., 2011) but the current data extend this to show a parallel upregulation in cytokine mRNA and to show that the LPS-induced increase in mRNA expression of CD40, ICAM-1 and CD11b, which indicate microglial activation, was greater in $CD200^{-/-}$ mice. Even under resting conditions, CD11b mRNA, although not ICAM-1 or CD40, was increased in glia prepared from $CD200^{-/-}$, compared with wildtype, mice, which might affect macrophage/microglial cell migration or cell-cell adhesion (Solovjov et al., 2005). Interestingly, retinal macrophage infiltrates have been observed in CD200-deficient mice with EAU (Broderick et al., 2002) and we have recently observed increased macrophage infiltration into the brain of these animals (Denieffe et al., unpublished). Whereas an increase

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in mRNA expression is not always paralleled by an increase in protein, we have previously reported that increased CD40 mRNA and CD11b mRNA expression in mixed glia prepared from CD200-deficient, compared with wildtype, mice was paralleled by increased staining when analysed by flow cytometry (Costello et al., 2011). This suggests that, at least in the case of these markers, a close correlation between mRNA expression and cell surface expression of the protein was observed.

We have attributed the increased responsiveness to LPS in CD200$^{-/-}$ mice to an increase in expression of TLR4 (Costello et al., 2011). Interestingly, cells prepared from CD200$^{-/-}$ mice are also more responsive to the TLR2 agonist Pam_3CSK_4 and this may be explained by the observed increased expression of TLR2 on cells prepared from CD200$^{-/-}$ mice, even under resting conditions (Costello et al., 2011). This enhanced reactivity of immune cells from CD200$^{-/-}$ mice to an inflammatory challenge *in vitro* corroborates reports by other groups that investigated inflammatory insults *in vivo*. Studies examining the susceptibility of CD200$^{-/-}$ mice to experimentally-induced allergic disease or viral infection revealed increased responsiveness to these inflammatory stimuli (Campbell et al., 2000, Hoek et al., 2000, Snelgrove et al., 2008). Additionally the onset of EAE was dramatically accelerated in CD200$^{-/-}$ mice, and the increase in iNOS- and CD68- positive macrophages appeared earlier and persisted for longer in the spinal cord of these mice (Hoek et al., 2000). Disease kinetics of EAU in CD200$^{-/-}$ mice displayed a similar course to that of EAE, with an earlier onset and increased severity of symptoms (Copland et al., 2007) and the inflammatory response following facial nerve transaction was also enhanced in these animals (Hoek et al., 2000). Consistent with the importance of CD200 in moderating microglial activation and inflammatory changes, we have recently reported that CD200Fc attenuates the age-related and LPS-induced increase in microglial activation and, concurrently, partially overcomes the deficits in LTP observed in aged rats and LPS-treated rats (Cox et al., 2012). Interestingly

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CD200 expression is decreased in several brain areas in post-mortem tissue obtained from patients with Alzheimer's disease (Walker et al., 2009). Although receptor expression is unchanged, induction of CD200R expression is impaired in monocyte-derived macrophage obtained from PD patients (Luo et al.) and it has been proposed that this may reflect the situation in microglia and therefore partially explain the increased microglial activation associated with the disease (Wang et al., 2007). These findings, as well as those from preclinical studies suggest that CD200R may be a useful target for therapeutics in neuroinflammatory conditions.

Work from this laboratory has revealed the expression of CD200 on neurons *in vitro* and identified that the immunomodulatory effect of neurons on glial cells in culture was CD200-dependent (Lyons et al., 2007). This proclivity is shared by endothelial cells (Miller et al., 2011) and, interestingly, both neurons and endothelial cells attenuated the effect of LPS in cultured glia. The current study demonstrated the presence of CD200 on CD11b-negative cells indicating that CD200 is expressed on astrocytes as previously described (Costello et al., 2011, Ojo et al., 2012) and show that addition of an astroglial membrane preparation to microglial cultures attenuates the LPS-induced increases in cytokine production. Significantly incubating astrocytes in the presence of FGL enhanced expression of CD200 and therefore FGL may exert its anti-inflammatory action *in vivo* by enhancing astrocytic, as well as neuronal CD200, expression (Downer et al., 2010).

It has been suggested previously that astrocytes can modulate microglial activity by enhancing or dampening their activation state through release of pro- or anti-inflammatory cytokines (Eng et al., 2000); the present data provide another mechanism by which control can be exerted suggesting that, in addition to soluble factors, astrocytes can modulate microglial activity by cell:cell contact, involving CD200-CD200R interaction.

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FIGURE LEGENDS

Figure 1. FGL attenuated the LPS-induced increase in CD40, ICAM-1and CD11b mRNA in glia prepared from wildtype but not $CD200^{-/-}$ mice.

LPS induced a significant increase in mRNA expression of CD40 (A), ICAM-I (B) and CD11b (C) in mixed glia cultured from wildtype and $CD200^{-/-}$ mice ($^{***}p<0.001$; ANOVA; A, $F_{(7,32)}=7.91$, B, $F_{(7,36)}=31.2$ and C, $F_{(7,34)}=60.95$;) and these responses were enhanced in glia from $CD200^{-/-}$mice ($^{\S}p<0.05$; §§ $p<0.01$; ANOVA). Pre-treatment with FGL attenuated the LPS-induced changes in glia from wildtype, but not $CD200^{-/-}$, mice ($^{+++}p<0.001$; ANOVA). Values are present as means (±SEM; n=6) expressed as a ratio to β-actin mRNA and standardised to a control sample.

Figure 2. FGL attenuated the LPS-induced increase in IL-1β mRNA and release in glia prepared from wildtype but not $CD200^{-/-}$ mice.

LPS induced a significant increase in IL-1β mRNA expression (A) and release (B) in mixed glia cultured from wildtype and $CD200^{-/-}$ mice, ($^{***}p<0.001$; ANOVA; A, $F_{(7,25)}=83.24$ and B, $F_{(7,35)}=7.77$). Pre-treatment with FGL attenuated the increase in IL-1β expression in glia from wildtype, but not $CD200^{-/-}$, mice ($^{+++}p<0.001$; ANOVA). The response to LPS was markedly enhanced in glia cultured from $CD200^{-/-}$, compared with cells prepared from wildtype, mice ($^{\S\S\S}p<0.001$; ANOVA). Values for Q-PCR are presented as means (±SEM; n=6) expressed as a ratio to β-actin mRNA and standardised to a control sample. Values for cytokine release are presented as means (±SEM; n=6) and expressed as pg of IL-1β/ml.

Figure 3. FGL attenuated the LPS-induced increase in TNF-α mRNA and release in glia prepared from wildtype but not $CD200^{-/-}$ mice.

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LPS induced a significant increase in TNF-α mRNA expression (A) and release (B) in mixed glia cultured from wildtype and CD200$^{-/-}$ mice, ($^{**}p<0.01$; $^{***}p<0.001$; ANOVA; A, $F_{(7,34)}=54.05$ and B, $F_{(3,12)}=73$). Pre-treatment with FGL attenuated the increase in TNF-α expression in glia from wildtype, but not CD200$^{-/-}$, mice ($^{++}p<0.01$; $^{+++}p<0.001$; ANOVA). The response to LPS was markedly enhanced in glia cultured from CD200$^{-/-}$, compared with cells prepared from wildtype, animals ($^{§§§}p<0.001$; ANOVA). Values for Q-PCR are presented as means (±SEM; n=6) expressed as a ratio to β-actin mRNA and standardised to a control sample. Values for cytokine release are presented as means (±SEM; n=6) and expressed as pg of TNF-α/ml.

Figure 4. FGL attenuated the LPS-induced increase in IL-6 mRNA and release in glia prepared from wildtype but not CD200$^{-/-}$ mice.

LPS induced a significant increase in IL-6 mRNA expression (A) and release (B) in mixed glia cultured from both mouse strains ($^{***}p<0.001$; ANOVA; A, $F_{(7,27)}=30.51$ and B, $F_{(7,34)}=1877$). Pre-treatment with FGL attenuated the increase in IL-6 expression in glia from wildtype, but not CD200$^{-/-}$, mice ($^{+++}p<0.001$; ANOVA). The response to LPS was markedly enhanced in glia cultured from CD200$^{-/-}$, compared with cells prepared from wildtype, animals ($^{§§§}p<0.001$; ANOVA). Values for Q-PCR are presented as means (±SEM; n=6) expressed as a ratio to β-actin mRNA and standardised to a control sample. Values for cytokine release are presented as means (±SEM; n=6) and expressed as pg of IL-6/ml.

Figure 5. CD200 expression is increased by FGL

A. Flow cytometric analysis indicate that CD200 was expressed on CD11b-negative cells in mixed glia cultured from wildtype mice (blue), but not CD200$^{-/-}$ mice (red). B. FGL

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significantly increased CD200 in isolated astrocytes prepared from wildtype mice ($^{*}p<0.05$; Student's *t*-test, densitometric units equalised to β-actin).

Figure 6. LPS induced an increase in IL-1β mRNA expression in isolated microglia. Pre-incubation with an astrocytic membrane fraction attenuated the LPS-induced changes.

A. LPS induced a significant increase in IL-1β mRNA expression in isolated microglia, ($^{**}p<0.01$; $^{***}p<0.001$; ANOVA; $F_{(4,17)}=16.01$). Pre-treatment with an astrocytic membrane fraction attenuated the increase in IL-1β mRNA expression in isolated microglia ($^{+++}p<0.001$; ANOVA). B,D. LPS induced a significant increase in mRNA expression of TNF-α and IL-6 ($^{**}p<0.01$; $^{***}p<0.001$; $F_{(7,35)}=7.77$, $F_{(3,21)}=5.63$ respectively. Pre-treatment with a membrane fraction prepared from astrocytes significantly attenuated the LPS-induced increases in TNF-α and IL-6 mRNA expression ($^{+}p<0.05$; $^{++}p<0.01$ANOVA). C,E. LPS induced a significant increase in supernatant concentrations of TNF-α and IL-6 ($^{**}p<0.01$; $^{***}p<0.001$; $F_{(3,14)}=18.27$, $F_{(3,17)}=6.68$ respectively). Pre-treatment with a membrane fraction prepared from astrocytes significantly attenuated the LPS-induced changes ($^{+}p<0.05$; $^{+++}p<0.01$; ANOVA). Values for Q-PCR are presented as means (±SEM; n=6) expressed as a ratio to β-actin mRNA and standardised to a control sample. Values for cytokine release are presented as means (±SEM; n=6) and expressed as pg TNF-α /ml.

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CONTRIBUTIONS OF AUTHORS

FFC carried out the experiments, VB and EB prepared the FGL, ML designed the study and wrote the article.

DISCLOSURE

E. Bock and V. Berezin are shareholders of ENKAM Pharmaceuticals A/S, which owns the FGL peptide (less than 0.01% shares each). This does not alter our adherence to all the Journal policies on sharing data and materials.

All authors have read and approved the final article.

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Figure 1

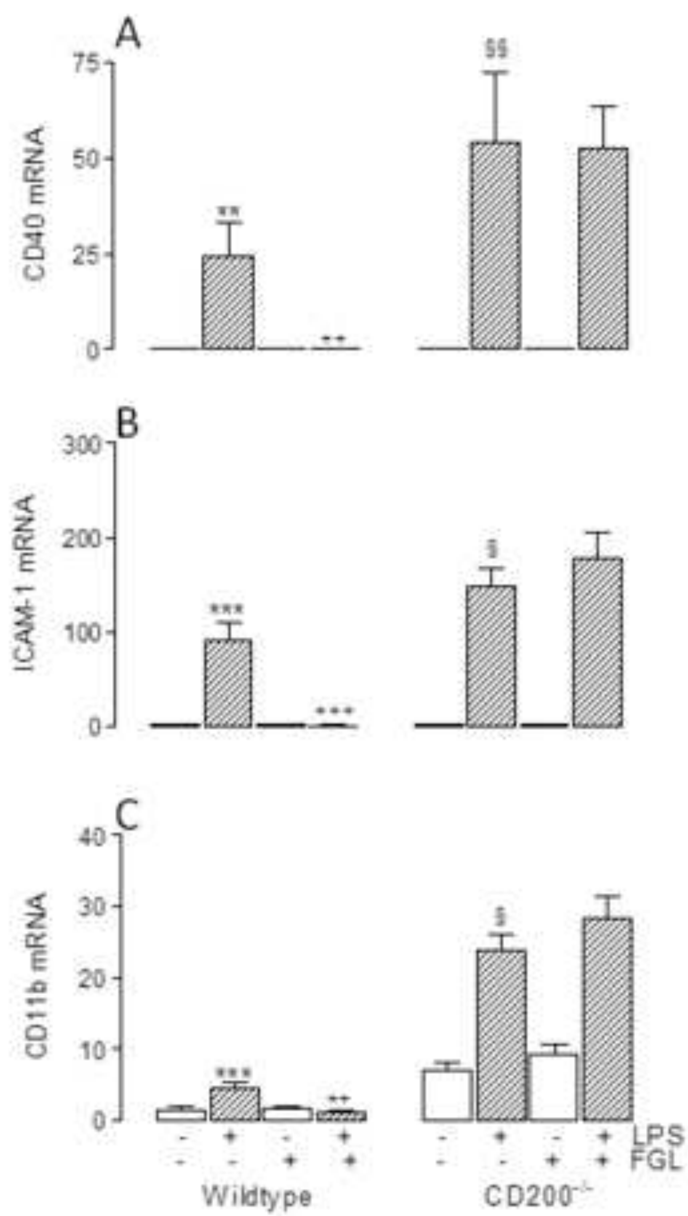

Figure 2

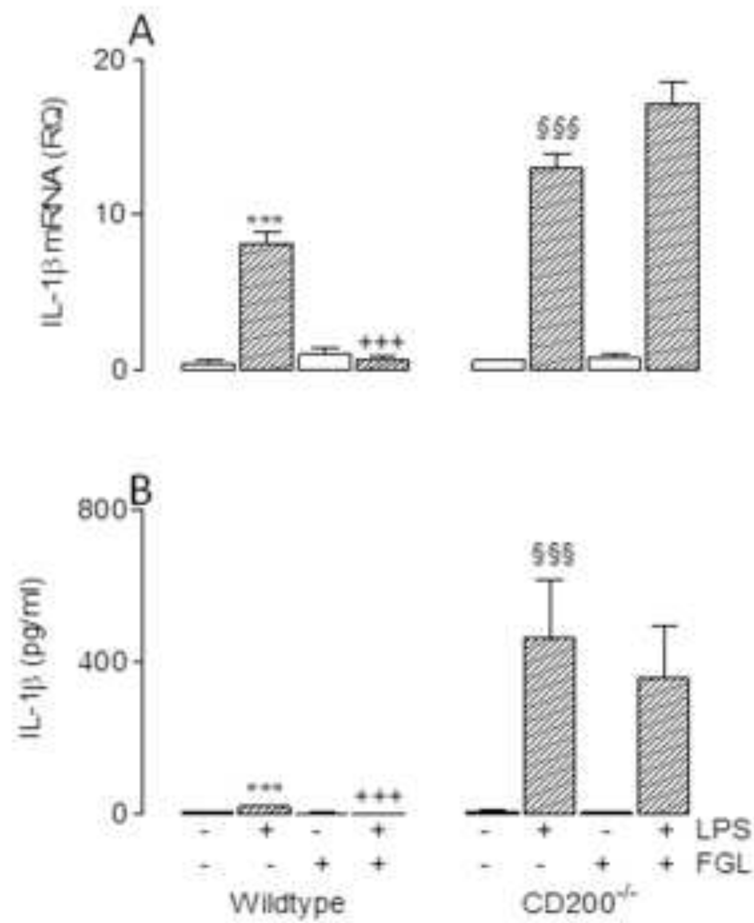

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Figure 3

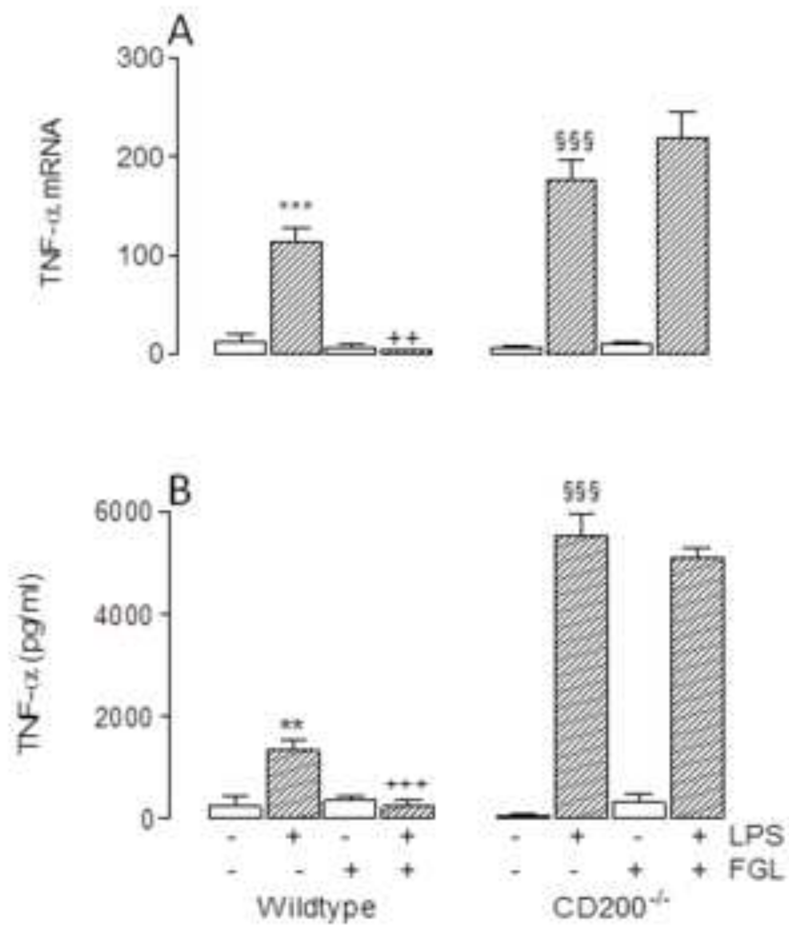


Figure 4

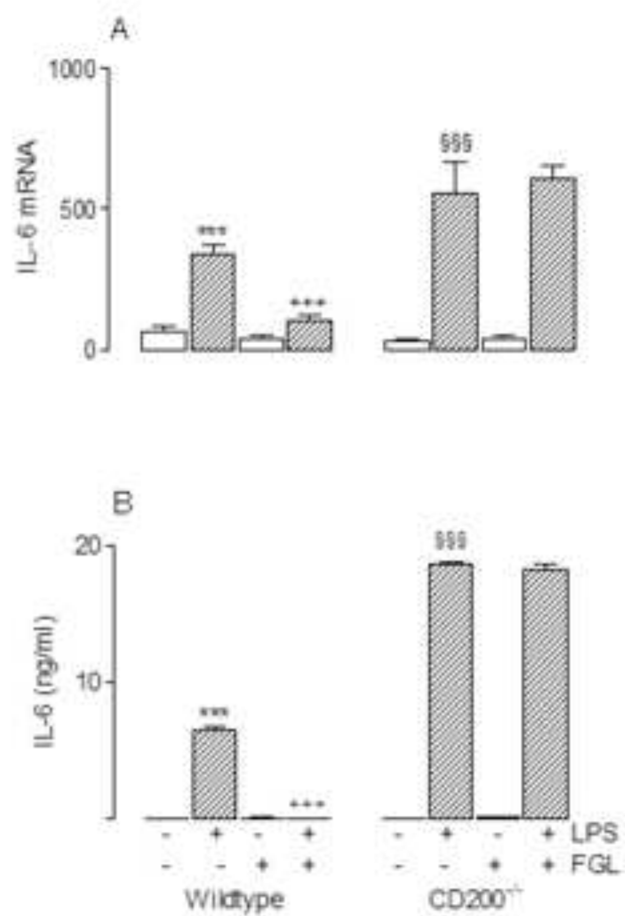


Figure 5

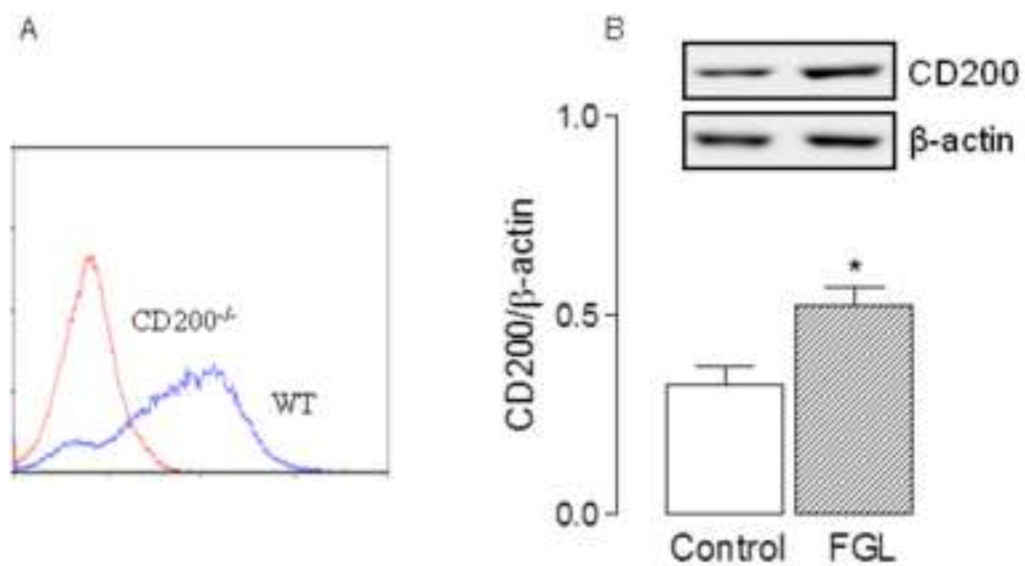
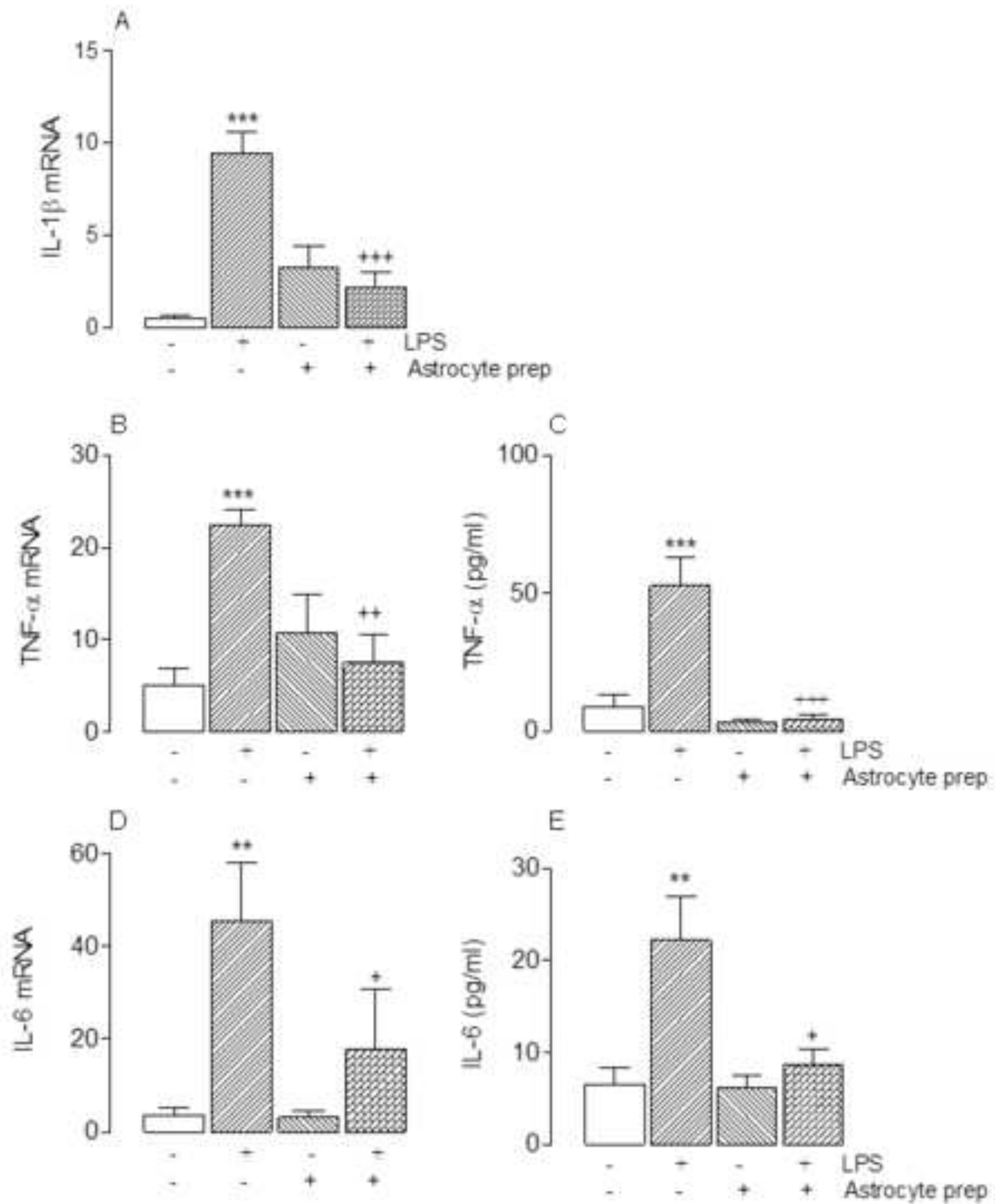


Figure 6



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HIGHLIGHTS

- LPS increased microglial activation and production of inflammatory cytokines in cultured cells
- The LPS-induced changes were greater in cells from CD200$^{-/-}$, compared with wildtype, mice
- FGL attenuated the LPS-induced changes in cells from wildtype, but not CD200$^{-/-}$, mice
- Astrocytes, which express CD200, play an important role in modulating microglial activation

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