The membrane glycoprotein CD200 is expressed on several cell types, including neurons, whereas expression of its receptor, CD200R, is restricted principally to cells of the myeloid lineage, including microglia. The interaction between CD200 and CD200R maintains microglia and macrophages in a quiescent state; therefore, CD200-deficient mice express an inflammatory phenotype exhibiting increased macrophage or microglial activation in models of arthritis, encephalitis, and uveoretinitis. Here, we report that lipopolysaccharide (LPS) and activation in models of arthritis, encephalitis, and uveoretinitis phenotype exhibiting increased macrophage or microglial state; therefore, CD200-deficient mice express an inflammatory phenotype exhibiting increased macrophage or microglial activation in models of arthritis, encephalitis, and uveoretinitis. Here, we report that lipopolysaccharide (LPS) and activation in models of arthritis, encephalitis, and uveoretinitis.

CD200 is a type-1 membrane glycoprotein which has been identified as an immunosuppressive molecule, consistent with its expression on cells of the immune system, including dendritic cells, T and B cells, and endothelial and epithelial cells (1). Diverse immunomodulatory roles for CD200 have been reported; these include antigen-specific T cell responses, suppression of regulatory T cells (2), cytotoxic T cell-mediated suppression of regulatory T cells (2), cytotoxic T cell-mediated suppression of regulatory T cells (2), cytotoxic T cell-mediated impact on synaptic plasticity.

The interaction between CD200 and CD200R maintains microglia and macrophages in a quiescent state; therefore, CD200-deficient mice express an inflammatory phenotype exhibiting increased macrophage or microglial activation in models of arthritis, encephalitis, and uveoretinitis. Here, we report that lipopolysaccharide (LPS) and activation in models of arthritis, encephalitis, and uveoretinitis phenotype exhibiting increased macrophage or microglial state; therefore, CD200-deficient mice express an inflammatory phenotype exhibiting increased macrophage or microglial activation in models of arthritis, encephalitis, and uveoretinitis.

In CD200-deficient mice, increased microglial and/or macrophage activation has been described in several models of inflammation (e.g. facial nerve transsection, experimental autoimmune encephalomyelitis, an animal model of arthritis (9), and experimental autoimmune uveoretinitis (10)). Conversely, administration of a CD200 fusion protein ameliorates the inflammatory changes observed in collagen-induced arthritis (11, 12), whereas the decrease in experimental autoimmune encephalomyelitis-like symptoms in Wld^mice has been attributed to increased expression of CD200 on spinal cord neurons (13).

Reduced expression of CD200 is coupled with increased microglial activation in hippocampus of aged and β-amyloid (Aβ)3-treated rats (8, 14), and synaptic plasticity, specifically long term potentiation (LTP), is impaired when microglial activation is increased (15, 16). Therefore, we predicted that glia prepared from CD200-deficient mice would respond more profoundly to LPS and that this would be coupled with evidence of impaired LTP. The data show that LPS and Pam3CSK4 exert a greater effect on glia prepared from CD200-deficient mice, presumably due to the observed increase in expression of TLR2 and TLR4 on these cells. In addition, LTP was markedly reduced at

3 The abbreviations used are: Aβ, β-amyloid; LTP, long term potentiation; Pam3CSK4, Pam3CysSerLys4; EPSP, excitatory postsynaptic potential; TLR, Toll-like receptor; TBS, θ-burst stimulation; ANOVA, analysis of variance; PE, phycoerythrin; GFAP, glial fibrillary acidic protein; GLAST, L-glutamate/L-aspartate transporter.
CA1 synapses of hippocampal slices prepared from CD200−/−, compared with wild type, mice. LPS and Pam3Csk4 further attenuated LTP in slices prepared from CD200−/− mice. The data provide further evidence for an important immunomodulatory role for CD200 and couple the loss of CD200 with a deficit in synaptic function and with increased expression of TLR2 and -4.

**EXPERIMENTAL PROCEDURES**

**Animals**—1-day-old and 2–6-month-old C57BL/6 or CD200−/− mice were used for preparation of glial cultures or for preparation of hippocampal slices, respectively. Tissue from 2–6-month-old mice was also used for analysis of expression of TLR2 and -4. In a second series of experiments, cells were harvested for flow cytometric analysis to evaluate expression of cell surface markers, FITC-rat anti-mouse TLR4 (Cambridge Biosciences), PE-rat anti-mouse CD200R (Serotec), FITC-rat anti-mouse TLR2 (Cambridge Biosciences), PE-Cy7-anti-mouse CD45 (BD Biosciences), and allophycocyanin-rat anti-mouse CD11b (BD Biosciences). We used mixed glia cultures contained 70% astrocytes and 30% microglia as assessed by expression of CD11b using FACS. We used mixed glia because CD200 is expressed on astrocytes but not microglia. This means that knocking out CD200 will have no impact on microglia unless they are in culture with astrocytes, and, in this case, the effect can be attributed to the loss of signaling through CD200R. In the context of this study, isolated microglia prepared from wild type and CD200−/− are essentially the same.

In one series of experiments, cells were harvested for flow cytometric analysis to evaluate expression of cell surface markers of microglial activation, for GLAST to identify astrocytes, or for PCR to evaluate expression of TLR2 and -4. In a second series of experiments, cells were incubated in the presence or absence of LPS (100 ng/ml; Alexis Biochemical) or Pam3Csk4 (100 ng/ml; InvivoGen), and, 24 h later, supernatant was collected and assessed for concentration of IL-1β, IL-6, and TNFα.

Purified astrocytes were prepared as described previously, using the shaking method to remove microglia (17), and membranes were isolated using a subcellular protein fractionation kit (Thermo Scientific). Cells were incubated in trypsin-EDTA (1 ml, 15 min, 37 °C), centrifuged (500 × g, 5 min), washed with ice-cold PBS, resuspended in PBS, and centrifuged (500 × g, 5 min). The pellet was resuspended in ice-cold Cytoplasmic Extraction Buffer containing protease inhibitors (Thermo Scientific), incubated (4 °C, 10 min), and centrifuged (3,000 × g, 5 min); the supernatant provided the cytosolic fraction, whereas the pellet, which contained the membrane fraction, was resuspended in ice-cold Membrane Extraction Buffer containing protease inhibitors (Thermo Scientific), incubated (4 °C, 10 min), and centrifuged (3,000 × g, 5 min). The resultant supernatant provided the membrane fraction.

To prepare microglia, cells were initially seeded onto 25-cm² flasks, and, after 24 h, medium was replaced with cDMEM containing GM-CSF (10 ng/ml) and M-CSF (20 ng/ml). After 10–14 days in culture, non-adherent microglia were harvested by shaking (110 rpm, 2 h, room temperature), tapping, and centrifuging (2,000 rpm, 5 min). The pellet was resuspended in cDMEM, and the microglia were plated onto 24-well plates at a density of 1 × 10⁵ cells/ml and maintained at 37 °C in a 5% CO₂ humidified atmosphere for up to 3 days prior to treatment.

**Flow Cytometry**—Glial cells were trypsinized (0.25% trypsin-EDTA; Sigma) and washed three times in FACS buffer (2% FBS, 0.1% NaN₃ in PBS). Whole brain tissue was harvested and passed through a cell strainer (70 μm) and centrifuged (170 × g, 10 min). The pellet was resuspended in PBS containing collagenase D (1 mg/ml) and DNase I (200 μg/ml), incubated at 37 °C for 30 min, and centrifuged (170 × g, 5 min). Pellets were resuspended in 1.088 g/ml Percoll (9 ml), underlayered with 1.122 g/ml Percoll (5 ml), and overlaid with 1.072 and 1.030 g/ml (9 ml each) Percoll and PBS (9 ml) and centrifuged (1,250 × g, 45 min). The mononuclear cells (between 1.088:1.072 g/ml and between 1.072:1.030 g/ml) were centrifuged, and the pellets were washed. All cells were blocked for 15 min at room temperature in FACS block (Mouse BD Fc Block (BD Pharmingen); 1:500 in FACS buffer). Cells were incubated with PE-Cy7- or allophycocyanin-rat anti-mouse CD11b (BD Biosciences), FITC-rat anti-mouse CD40 (BD Biosciences), PE-rat anti-mouse MHCII (BD Biosciences), allophycocyanin-rat anti-mouse CD200 (BD Biosciences), PE-rat anti-mouse CD200R (Serotec), FITC-rat anti-mouse TLR2 (Cambridge Biosciences), FITC-rat anti-mouse TLR4 (Cambridge Biosciences), PE-Cy7-anti-mouse CD45 (BD Biosciences), and allophycocyanin-rat anti-mouse GLAST (BD Biosciences). Antibodies were diluted (1:400) in FACS buffer. Immunofluorescence analysis was performed on a DAKO Cyan ADP 7 color flow cytometer (DAKO Cytomation) with Summit version 4.3 software.

**Real-time PCR Analysis**—Total RNA was extracted from snap-frozen hippocampal tissue and harvested mixed glial cells using a NucleoSpin® RNAII isolation kit (Macherey-Nagel Inc.), and cDNA synthesis was performed on 1 μg of total RNA using a High Capacity cDNA RT kit (Applied Biosystems); the protocols used were according to the manufacturer’s instructions. Real-time PCR was performed as described previously (8) using an ABI Prism 7300 instrument (Applied Biosystems). The primer IDs were as follows: CD11b, Mm01271265_m1; CD40, Mm00445273_m1 (Applied Biosystems). Samples were assayed in duplicate, and gene expression was calculated relative to the endogenous control samples (β-actin) to give a relative quantity value (2−ΔΔCt, where Ct is the threshold cycle).

**Analysis of IL-1β, IL-6, and TNFα**—The concentrations of IL-1β, IL-6, and TNFα were analyzed in triplicate by ELISA in samples of supernatant obtained from in vitro experiments as described previously (8).

**Analysis of CD200, GFAP, plkBα, IL-1α, IL-1β, and TNFα by Western Immunoblotting**—Hippocampal lysate was assessed for expression of IL-1α, IL-1β, and TNFα; glial cell lysate was evaluated for expression of plkBα; and membrane and cytosolic preparations obtained from purified astrocytes were evaluated for expression of CD200 and GFAP using standard Western immunoblotting methods (8, 18). Primary antibodies directed against CD200 (anti-goat; 1:500; Santa Cruz Biotechnology,
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Inc., Santa Cruz, CA), GFAP (anti-rabbit; 1:1000; Invitrogen), pIk-B (Ser-32) (anti-rabbit; 1:1000; Cell Signaling), IL-1α (anti-goat; 1:1000; R&D Systems), IL-1β (anti-goat; 1:500; Santa Cruz Biotechnology, Inc.), and TNFα (anti-rabbit; 1:500; Cell Signaling) were incubated overnight at 4 °C. The secondary antibodies were conjugated to horseradish peroxidase (1:5000; Jackson Immunoresearch), and after a 2-h incubation step, membranes were washed, and protein complexes were visualized (Immobilon Western chemiluminescent substrate, Millipore). Membranes were stripped and probed for β-actin (to confirm equal loading) as described previously (8, 18). Images were captured using the Fujifilm LAS-3000 imager, and densitometric analysis was used to quantify expression of the proteins. Values are presented as mean ± S.E., normalized to β-actin.

Hippocampal Slice Preparation and LTP Recording—Acute hippocampal slices (400 μm) from C57BL/6 and CD200\textsuperscript{−/−} mice were prepared using a McIlwain tissue chopper as described previously (18) and maintained in oxygenated artificial cerebrospinal fluid (125 mM NaCl, 1.25 mM KCl, 2 mM CaCl\textsubscript{2}, 1.5 mM MgCl\textsubscript{2}, 1.25 mM KH\textsubscript{2}PO\textsubscript{4}, 25 mM NaHCO\textsubscript{3}, and 10 mM D-glucose) at room temperature (21–23 °C) in a holding chamber for a minimum of 1 h before being transferred to a submersion recording chamber. Slices were continuously perfused (2–3 ml/min) with oxygenated artificial cerebrospinal fluid at room temperature (21–23 °C). The Schaffer collateral-commissural pathway was stimulated at 0.033 Hz (0.1-ms duration of LTP recording.)

In hippocampal slices prepared from wild type and CD200\textsuperscript{−/−} mice. In addition, there was no evidence of epileptiform activity in any slice. Stable base line EPSPs were recorded for 20 min prior to application of θ-burst stimulation (TBS; 10 trains (4 pulses at 100 Hz) repeated at 5 Hz (18)). In some experiments, LPS (Alexis Biochemicals) or Pam\textsubscript{3}C\textsubscript{rk} (InvivoGen) was added to the perfusate (10 μg/ml for 20 min or 20 μg/ml for 60 min) prior to TBS. In an additional set of experiments, slices were perfused with mouse recombinant TNFα (R&D Systems; 3 ng/ml in 0.002% BSA) or vehicle alone (0.002% BSA), for 20 min prior to LTP induction. This concentration of TNFα is less than that previously demonstrated to impair LTP in hippocampal slices prepared from rats (19, 20).

Data were acquired using WinWCP version 4.0.7 software (Dr. J. Dempster, Strathclyde, UK). Evoked EPSPs were normalized to the slope recorded in the 5-min period prior to LTP induction. The level of LTP was evaluated as the mean percentage EPSP slope during the last 5 min of recording, and data are presented as mean percentage EPSP slope ± S.E.

Hippocampal slices not used for electrophysiology were prepared for Western immunoblot analysis of IL-1α, IL-1β, and TNFα expression (described above). These slices were incubated as described for a minimum of 1 h following the slicing procedure plus a further incubation period equivalent to the duration of LTP recording.
membrane, but not cytosolic, fractions (Fig. 3), whereas GFAP expression was, predictably, largely confined to the cytosolic fractions.

A possible explanation for the increase in responsiveness of cells from CD200/−/− mice to LPS and Pam3Csk4 is the significant increase in expression of both TLR2 and TLR4 mRNA in mixed glia prepared from CD200/−/− compared with wild type mice (*, p < 0.05; Student’s t test for independent means; Fig. 4, a and d). Flow cytometric analysis demonstrated that cell surface expression of both receptors was increased on CD11b+ cells obtained from CD200/−/− compared with wild type mice, but the increase was significant only in the case of TLR2 (**, p < 0.01; Student’s t test for independent means; Fig. 4, b, c, e, and f).

The significant increase in phosphorylated IκBα in cells prepared from CD200/−/− compared with wild type mice (*, p < 0.05; Student’s t test for independent means; Fig. 4g) indicates that signaling through TLR is up-regulated in cells prepared from CD200/−/− mice.

CD200 deficiency is accompanied by inflammatory changes (9, 10), and, in the brain, microglial activation is coupled with decreased CD200 in brains of aged animals and also in LPS-treated and Aβ-treated animals (8, 21). To investigate this correlation further, we evaluated expression of surface markers of microglial activation on cells prepared from CD200/−/− and wild type mice using PCR and flow cytometry and show that CD40 mRNA, but not CD11b mRNA, was significantly increased in tissue prepared from CD200/−/− compared with wild type mice (*, p < 0.05; Student’s t test for independent means; Fig. 5, a and b). Analysis by flow cytometry indicated that there was no genotype-related change in CD11b+ cells (Fig. 5c), but the percentage of CD11b+ cells that were positive for MHCII and CD40 was significantly increased (*, p < 0.05; ***, p < 0.001; Student’s t test for independent means; Fig. 5, d–g).

CD45 has been used as a means of discriminating between macrophages (which express high levels of CD45) and microglial (which express low levels of CD45 (22)). Flow cytometric analysis revealed that the numbers of CD11b+ CD45low cells were significantly increased in hippocampus of CD200/−/− compared with wild type mice (***, p < 0.001; Student’s t test for independent means; Fig. 6a) and that CD200R expression (b), CD40 (c), TLR2 (d), and TLR4 (f) on these cells was greater in tissue prepared from CD200/−/− compared with wild type mice. The numbers of macrophages in the brain (i.e. CD11b+ CD45high cells) were negligible in CD200/−/− and wild type mice. Analysis of expression of TLR in hippocampus revealed
that both TLR2 and -4 were increased in CD200−/− compared with wild type mice (*, p < 0.05; **, p < 0.01; Student’s t test for independent means; Fig. 6, e and g). These changes indicate that microglial activation occurs in brain tissue of CD200−/− mice, and therefore the changes in vitro are reflected in vivo, although the increase in expression of TLR2 mRNA in hippocampus is markedly greater than the change observed in cultured cells. Significantly, this was accompanied by a deficit in LTP in CA1 synapses where the response, 60 min following application of TBS, was markedly reduced in slices prepared from CD200−/− mice (12 slices from seven mice) compared with wild type mice (15 slices from 11 mice; p < 0.001; unpaired Student’s t test; Fig. 6h). Although a number of inflammatory cytokines released from activated microglia might exert this effect (17–21), here we show that whereas expression of IL-1α and IL-1β were similar in hippocampal tissue prepared from wild type and CD200−/− mice (Fig. 7, a and b), TNFα was increased (p < 0.05; Student’s t test for independent means; Fig. 7c). As previously demonstrated in hippocampal slices prepared from rats (19, 20, 23), application of TNFα (3 ng/ml) to mouse hippocampal slices significantly impaired LTP relative to vehicle controls (p < 0.05; unpaired Student’s t test; three slices from two mice; Fig. 7d).

Because cells prepared from CD200−/− mice showed increased susceptibility to LPS, we predicted that concentrations of LPS that exerted no effect on LTP in wild type mice may attenuate it in CD200−/− mice. Application of LPS (20 μg/ml) to hippocampal slices from wild type mice for 60 min prior to TBS inhibited LTP (five slices from five mice) compared with controls (15 slices from 11 mice; p < 0.001; Fig. 8a). In contrast, a lower concentration of LPS (10 μg/ml; 20-min pretreatment), which exerted no effect on LTP in slices prepared from wild type mice (seven slices from six mice; Fig. 8b), significantly decreased LTP in slices from CD200−/− mice (13 slices from nine mice) relative to control (12 slices from seven mice; p < 0.05; Fig. 8c).

Like LPS, Pam3Csk4 exerted a greater effect on inflammatory markers in cells prepared from CD200−/− mice, and therefore we predicted that its effect on LTP would be genotype-specific. Application of Pam3Csk4 (20 μg/ml) to hippocampal slices pre-
pared from wild type mice for 60 min prior to TBS inhibited LTP (three slices from three mice) compared with untreated controls (15 slices from 11 mice; \( p < 0.001; \) Fig. 9a). A lower concentration of Pam3Csk4 (10 \( \mu \)g/ml), applied for 20 min prior to TBS, did not affect LTP in slices prepared from wild type mice (four slices from three mice; Fig. 9b) but significantly reduced LTP in slices prepared from CD200\(^{-/-}\) mice (six slices from five mice) compared with control (12 slices from seven mice; \( p < 0.05; \) Fig. 9c).

**DISCUSSION**

The loss of CD200 has a significant impact on activation of microglia in response to inflammatory stimuli, probably because of increased expression of TLR4 and TLR2 in vitro and in vivo. Whereas LTP in Schaffer collateral-CA1 synapses was markedly impaired in slices prepared from CD200-deficient mice under control conditions, activation of TLR4 and TLR2, by LPS and Pam3Csk4, respectively, exerted a more profound effect on LTP in slices prepared from CD200\(^{-/-}\) mice. We propose that the increased expression of TLR4 and TLR2 provides a plausible explanation for the increased responsiveness of CD200\(^{-/-}\) mice to inflammatory stimuli.

LPS and Pam3Csk4 increased the release of proinflammatory cytokines, IL-1\(\beta\), IL-6, and TNF\(\alpha\), from mixed glial cultures, confirming previously described effects of TLR4 and TLR2 (21, 24, 25). Both agonists exerted a greater effect on release of proinflammatory cytokines in mixed glia prepared from CD200\(^{-/-}\) mice, compared with wild type mice. Thus, tonic activation of CD200 receptor by CD200 is required to modulate inflammatory cytokine production. This concurs with data indicating that the interaction of neurons and microglia by means of CD200 receptor engagement by CD200 decreased microglial activation and production of IL-1\(\beta\) (8). In the current study, in which a mixed glial preparation was used, we propose that the modulating effect is a consequence of the interaction between microglia and astrocytes, which we demonstrate express CD200. It is known that CD200 is widely expressed on numerous cell types, although, in the case of astrocytes, expres-
sion to date has been reported only on reactive astrocytes in lesions from post-mortem brains of individuals with multiple sclerosis (7). An interesting possibility is that the relatively activated state of microglia in a purified microglial culture may be a consequence of the loss of the CD200-controlled modulating effect of astrocytes.

The present findings in glia mirror those observed in peritoneal macrophages; thus, stimulation with LPS and peptidoglycan and also poly(I:C) increased release of TNFα and IL-6 to a greater extent in macrophages prepared from CD200−/− mice compared with wild type mice (26). Similarly, alveolar macrophages prepared from CD200−/− mice, when stimulated ex vivo with LPS or IFNγ, expressed more MHCII and released more inflammatory cytokines than macrophages from wild type mice (27). It has been known for many years that astrocytes are capable of modulating microglial/macrophage function. They have been shown to modulate LPS-induced changes in inducible nitric oxide synthase and NO production (28, 29) and expression of MHCII (30), effects that have been attributed to astrocytic release of soluble factors like transforming growth factor TGFβ. The present findings uncover another mechanism by which astrocytes can modulate microglial activation.

Several studies have established that responses to insults that induce inflammatory changes are exacerbated in CD200−/− mice. Thus, the symptoms and inflammation associated with experimental autoimmune encephalomyelitis, Toxoplasma encephalitis, experimental autoimmune uveoretinitis, collagen-induced arthritis, and facial nerve transaction are more profound in CD200-deficient mice (9, 10, 31). In addition, the response to an influenza dose of hemagglutination was much more severe (inducing some fatalities) in CD200-deficient compared with wild type mice (27). Although it has been shown that CD200R activation by a CD200Fc ameliorates the symptoms associated with these conditions and although CD200R-mediated regulation of macrophages relies on the binding of Dok2 to the PTB binding motif in the cytoplasmic region of CD200R and

FIGURE 5. Markers of microglial activation are increased in cells prepared from CD200−/− mice. a and b, expression of CD40 mRNA, but not CD11b mRNA, was significantly greater in mixed glia prepared from CD200−/− compared with wild type mice (*, p < 0.05; Student’s t test for independent means; n = 4–5). c–f, flow cytometric analysis revealed that the percentage of CD11b+ cells was similar in wild type and CD200−/− (c), but the percentage of CD11b+ cells that also stained positively for CD40 (d and e) and MHCII (f and g) was significantly greater in mixed glia obtained from CD200−/− compared with wild type mice (*, p < 0.05; ***, p < 0.001; Student’s t test for independent means; n = 4–8). Error bars, S.E.
the subsequent recruitment and activation of RasGAP (32), the mechanism by which these changes lead to dampening the activation of macrophage/microglia remains to be fully explained. In this study, we show that increased expression of both TLR4 and TLR2 was observed in glia prepared from CD200°/° mice, and this may, at least in part, provide an explanation for the susceptibility of CD200°/° mice to inflammatory stimuli. Both TLR2 and TLR4 ultimately lead to activation of NFκB, and, in this study, the increased receptor expression in glia prepared from CD200°/° mice is coupled with increased expression of phosphorylated IκB,
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FIGURE 7. Increased hippocampal expression of TNF\(\alpha\) in CD200\(^{-/-}\) mice may underlie the associated deficit in LTP. IL-1\(\alpha\) and IL-1\(\beta\) were similar in tissue prepared from wild type and CD200\(^{-/-}\) mice (a and b), but TNF\(\alpha\) was significantly increased (\(p < 0.05\); Student’s t test for independent means; c), as revealed by sample immunoblots and analysis of densitometric data. Application of TNF\(\alpha\) (3 ng/ml) to hippocampal slices significantly impaired LTP relative to vehicle controls (\(p < 0.05\); unpaired Student’s t test; three slices from two mice; d). Sample EPSP traces immediately prior to and 60 min following TBS are presented (scale bars, 1 mV/20 ms). Error bars, S.E.

which is indicative of NF\(\kappa\)B activation. These changes clearly provide one possible explanation for the increased responsiveness of these cells to LPS and Pam\(_3\)Csk\(_4\) in the present study and perhaps also in other models.

Loss of CD200 increases expression of markers of microglial activation in mixed glial cultures; CD200 deficiency was associated with enhanced expression of CD40 mRNA but not CD11b mRNA. In parallel, flow cytometry revealed that these markers and also MHCII were increased on CD11b-positive cells prepared from CD200\(^{-/-}\) mice. Previous studies have highlighted the importance of the interaction between CD200 and CD200R in maintaining the quiescent state of microglia and have revealed that the age-related and A\(\beta\)-induced increases in microglial activation are coupled with decreased CD200 expression on neurons (8, 14, 21). The present observations also concur with the findings that under resting conditions, spinal cord microglia adopt an inflammatory morphology expressing more CD11b (9), and the number of CD45\(^{+}\)CD11b\(^{+}\) cells prepared from retina of CD200\(^{-/-}\) mice was increased (10).

In the past decade, it has become clear that neuroinflammatory changes, coupled with increased microglial activation, negatively affect synaptic plasticity in aged, LPS-treated, and A\(\beta\)-treated rats (15, 33–35). These observations are corroborated in this study, where we directly associate the loss of CD200 with microglial activation and a deficit in LTP. The evidence indicates that slices prepared from CD200\(^{-/-}\) mice do not display LTP to the same degree as slices prepared from wild type mice. One possible explanation for this is that TNF\(\alpha\), which is increased in hippocampal tissue prepared from these mice, is released from activated microglia and inhibits LTP. We demonstrate that TNF\(\alpha\) inhibits TBS-induced LTP in mouse Schaffer collateral-CA1 synapses, which concurs with previous evidence indicating that it exerts a similar effect on tetanus-induced LTP in rats in vitro and in vivo (17, 19, 23).

In addition to the decrease in LTP observed in untreated slices prepared from CD200\(^{-/-}\) mice, the data indicate that a subthreshold concentration of LPS or Pam\(_3\)Csk\(_4\) which exerts minimal effects on LTP in wild type mice, markedly impairs LTP in slices prepared from CD200\(^{-/-}\) mice. These findings show for the first time that activation of TLR2 leads to inhibition of LTP and further emphasize the protective effect of CD200-CD200R interaction, such that a deficit in CD200 leads to increased susceptibility to inflammatory stimuli. At this
point, it is unclear whether the effects of LPS or Pam3Csk4 on LTP are secondary to changes in glia or are a consequence of a direct effect on neuronal TLR4 and TLR2. In this regard, it is important to note that although some groups have reported neuronal expression of most TLRs both in vitro and in vivo (36, 37), others have been unable to demonstrate expression of TLR2 on neurons (38). The implication of this finding for the present study is that the mechanism underlying the Pam3Csk4-induced depression in LTP may result from its ability to release IL-1β, IL-6, and TNFα from glia; each of these inflammatory cytokines has been shown to inhibit LTP (17, 39, 40).

Although there is an accumulating body of evidence indicating that CD200 deficiency is associated with increased inflammatory changes in several tissues, including the brain, the effect on neuronal function is relatively unexplored. Here we report that activation of TLR4 and TLR2 exacerbates neuroinflammatory changes in the absence of CD200 and, importantly, demonstrate that CD200 deficiency also exerts a negative effect on LTP. A key factor underlying these changes is increased expression of these receptors. The findings highlight the importance of CD200 as a potential therapeutic target in disorders that are characterized by neuroinflammatory changes, coupled with loss of synaptic function.

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