Microglia and Astrocyte Activation by Toll-Like Receptor Ligands: Modulation by PPAR-γ Agonists

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We performed a comprehensive survey on the efficacy of various PPAR-γ agonists to modulate proinflammatory mediator release from primary microglia and astrocytes in response to numerous TLR ligands relevant to CNS infectious diseases. The results demonstrated differential abilities of select PPAR-γ agonists to modulate glial activation. For example, 15d-PGJ2 and pioglitazone were both effective at reducing IL-12 p40 release by TLR ligand-activated glia, whereas CXCL2 expression was either augmented or inhibited by 15d-PGJ2, effects that were dependent on the TLR ligand examined. Pioglitazone and troglitazone demonstrated opposing actions on microglial CCL2 production that were TLR ligand-dependent. Collectively, this information may be exploited to modulate the host immune response during CNS infections to maximize host immunity while minimizing inappropriate bystander tissue damage that is often characteristic of such diseases.

1. INTRODUCTION

Microglia and astrocytes participate in the genesis of innate immune responses in the CNS parenchyma [1, 2]. Their strategic placement at or near the blood-brain barrier likely makes both glial types sentinel cells for surveying pathogen entry in the CNS parenchyma. Indeed, both microglia and astrocytes are capable of producing a wide range of proinflammatory mediators in response to a diverse array of microbial stimuli [3, 4]. Therefore, it is likely that resident glia are pivotal for eliciting a local CNS inflammatory response through the initial production of inflammatory mediators, which in turn, leads to the recruitment of additional immune effector cells from the peripheral circulation.

Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRRs) responsible for recognizing conserved motifs expressed on broad classes of microbes termed pathogen-associated molecular patterns (PAMPs) [5, 6]. Typically, PAMPs represent structural or nucleic acid motifs that are less likely to undergo mutation, ensuring efficient pathogen recognition with a limited receptor arsenal [7]. A total of 13 TLRs have been identified to date, each conferring recognition of conserved motifs from large subclasses of bacteria, viruses, yeast, and fungi [5–7]. In addition, recent evidence indicates that TLRs are also capable of sensing endogenous ligands produced during stress or injury referred to as danger-associated molecular patterns (DAMPs) that may participate in autoimmune induction [8–10]. Numerous TLRs have been identified on microglia and astrocytes and both glial types are responsive to numerous TLR ligands implicating their role in pathogen recognition (reviewed in [11, 12]). In addition, recent evidence links TLRs with the host response to CNS injury presumably via recognition of endogenous “danger signals” since classical microbial TLR ligands are not present (reviewed in [13–15]). Since TLRs have been implicated in both infectious and noninfectious diseases of the CNS (reviewed in [11, 13]), understanding their potential to influence the course
of neuroinflammation is paramount and under certain conditions inappropriate TLR activation may contribute to excessive tissue destruction. Therefore, modulating TLR activity to achieve optimal benefit for the host may be a plausible strategy for minimizing tissue damage during neuroinflammatory disorders.

A group of compounds with reported anti-inflammatory effects in several models of inflammation, including the CNS, are ligands that interact with peroxisome proliferator-activated receptor-gamma (PPAR-γ) [16–18]. PPAR-γ is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors that regulate the expression of genes involved in reproduction, metabolism, development, and immune responses [17, 19]. A wide array of both natural and synthetic agonists for PPAR-γ have been identified including the naturally occurring prostaglandin metabolite 15d-PGJ2, thiazolidinediones (TZDs) a group of synthetic PPAR-γ agonists used for the treatment of diabetes, polyunsaturated fatty acids, and certain highaffinity tyrosine derivatives. With regard to the CNS, several PPAR-γ agonists have been documented for their ability to attenuate both microglial and astrocyte activation in response to a diverse array of stimuli as well as impact the course of several neurodegenerative diseases [16, 18, 20–25]. Although we and others have demonstrated that select PPAR-γ agonists are potent inhibitors of TLR2 and TLR4 activation (PGN and others have demonstrated that select PPAR-γ agonists are potent inhibitors of TLR2 and TLR4 activation (PGN and LPS, resp.) [23, 24, 26–29], a comprehensive examination of the effects of PPAR-γ agonists on a wide array of TLR ligands is lacking. In addition, although several studies describing the responses of microglia and astrocytes to TLR ligands exist [30–34], no reports have systematically investigated the ability of PPAR-γ ligands to modulate glial activation in response to TLR signals. Therefore, the purpose of this study was to define the actions of a panel of PPAR-γ agonists on TLR ligand-induced activation of microglia and astrocytes. Although within the same class of compounds, not all PPAR-γ agonists shared similar regulatory properties in response to various TLR ligands. Indeed, in some cases, inflammatory mediator production was enhanced following PPAR-γ agonist treatment. Collectively, these results suggest selective actions of PPAR-γ agonists on glial responses to TLR ligands that could be exploited for specific neuroinflammatory/infectious conditions of the CNS.

2. MATERIALS AND METHODS

2.1. TLR ligands and PPAR-γ agonists

The following TLR agonists were used in this study (see Table 1) with the concentration of each and its TLR target identified in parenthesis: Pam3CSK4 (TLR2, 1 μg/ml), polyinosine-polycytidylic acid (polyI:C; TLR3, 25 μg/ml), lipopolysaccharide from E. coli O111:B4 (LPS; TLR4, 100 ng/ml), flagellin from Salmonella typhimurium (TLR5, 10 μg/ml), single-stranded RNA (ssRNA; TLR7/8, 10 μg/ml), and synthetic unmethylated CpG oligonucleotide (ODN; TLR9, 0.1 μM and 5 μM for microglia and astrocytes, resp.). All TLR ligands were obtained from InvivoGen (San Diego, Calif, USA).

The natural PPAR-γ agonist 15d-PGJ2 and synthetic TZDs ciglitazone, rosiglitazone, pioglitazone, and troglitazone were purchased from Cayman Chemical (see Table 2; PPAR-γ Pak; Ann Arbor, Mich, USA). Dose-response studies were performed for all TZDs (10, 30, and 100 μM) and 15d-PGJ2 (5, 10, and 20 μM) in both TLR-activated microglia and astrocytes.

2.2. Primary microglia and astrocyte cultures

Primary microglia and astrocytes were isolated from C57BL/6 pups (2 to 4 days of age) as previously described [35, 36]. The purity of glial cultures was evaluated by immunohistochemical staining using antibodies against CD11b (BD Pharmingen) and glial fibrillary acidic protein (GFAP, Invitrogen, Carlsbad, Calif, USA) to identify microglia and astrocytes, respectively. The purity of primary microglia and astrocyte cultures was approximately 98% and 95%, respectively.

Throughout this study, microglia and astrocytes were seeded into 96-well plates at 2 × 10^4 or 1 × 10^5 cells/well, respectively, and incubated overnight. The following day, glia were pretreated with various PPAR-γ agonists for 1 hour prior to stimulation with the TLR ligand panel for 24 hours. Cell-conditioned supernatants were collected at 24 hours following TLR ligand treatment for quantitation of proinflammatory mediator expression by ELISA.

2.3. Cell viability assays

The effects of PPAR-γ and TLR agonists on glial cell viability were evaluated using a standard MTT assay based upon the mitochondrial conversion of (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) into formazan crystals. Results are reported as the raw OD_{570} values (mean ± SD).

2.4. Enzyme-linked immunosorbent assays (ELISAs)

Protein levels of TNF-α and CXCL2 (MIP-2) (Biosource) and IL-12 p40 and CCL2 (MCP-1, OptEIA, BD Pharmingen, San Jose, Calif, USA) were quantified in conditioned medium from PPAR-γ and TLR ligand-treated glia using ELISA kits according to the manufacturer’s instructions (level of sensitivity = 15.6 pg/ml).

2.5. Nitrite assay

Nitrite levels, a stable end product resulting from the reaction of NO with molecular oxygen, were determined in astrocytes by adding 50 μl of cell-conditioned culture medium with 50 μl of Griess reagent (0.1% naphthylethlenediamine dihydrochloride, 1% sulfanilamide, 2.5% phosphoric acid, all from Sigma) in a 96-well plate. The absorbance at 550 nm was measured on a plate reader (Spectra Max 190, Molecular Devices, Sunnyvale, Calif, USA), and nitrite concentrations were calculated using a standard curve with sodium nitrite (NaNO₂, Sigma, level of sensitivity, 0.4 μM). Based on our previous findings where S. aureus-derived TLR ligands were
potent inducers of NO in astrocytes but not microglia [24, 33, 34], we only quantitated NO levels in the former in the current study.

2.6. Statistics

Significant differences between experimental groups were determined by the Student’s t-test at the 95% confidence interval using SigmaStat (SPSS Science, Chicago, Ill, USA).

In this study, we performed a minimum of two independent replicates of each experiment to confirm the results obtained. The reporting of our results as representative of “x” number of independent experiments was required since it is difficult to achieve identical levels of proinflammatory mediator expression with distinct preparations of primary glia. As a result, the absolute concentrations of the various proinflammatory mediators differed between individual experiments; however, the trends were consistent. This required us to report results from a single study where each experimental treatment was represented by 3-4 individual wells (i.e., biological replicates) and statistical analysis conducted.

3. RESULTS

3.1. Ability of PPAR-γ agonists to modulate microglial cytokine production in response to diverse TLR ligands

Microglia represent the main innate immune effector in the CNS parenchyma as evident by their expression of numerous TLRs [32, 37, 38]. Although much emphasis has been placed on the neurodestructive properties of activated microglia, recent studies have revealed that in the correct context microglia can also facilitate CNS repair [39–41]. Therefore, striking the correct balance between regulated and inappropriate microglial activation may lead to optimal outcomes for a wide range of CNS neuroinflammatory conditions. To determine whether PPAR-γ agonists could serve to modulate microglial activation in the context of CNS infection, the effects of these compounds on microglial cytokine production in response to diverse TLR ligands were examined. The natural PPAR-γ agonist 15d-PGJ2 was uniformly found to inhibit IL-12 p40 release in response to all TLR ligands tested including Pam3Cys4, polyI:C, LPS, flagellin, ssRNA, and ODN (Figure 1). Fairly comprehensive reductions in IL-12 p40 production were also observed with all TLR ligands tested in response to the synthetic PPAR-γ agonists rosiglitazone (Figure 2) and pioglitazone (Figure 3) although the extent of inhibition was dramatically less compared to 15d-PGJ2. Rosiglitazone exhibited significant toxicity to primary microglia at the highest dose tested (i.e., 100 μM), hence it was not included in the final analysis. In contrast, ciglitazone did not dramatically affect IL-12 p40 production in response to the majority of TLR ligands examined (data not shown). Troglitazone exhibited microglial toxicity at the two highest doses of agonist tested (i.e., 100 and 30 μM); therefore, the results of this PPAR-γ agonist on microglial mediator production in response to TLR ligands are not presented.

| PPAR-γ agonist | Pam3Cys | polyI:C | LPS | Flagellin | ssRNA | ODN |
|----------------|---------|---------|-----|-----------|-------|-----|
| 15d-PGJ2       | ↑ IL-12 | ↑ IL-12 | ↓ IL-12 | ↑ IL-12 | ↑ IL-12 | ↓ IL-12 |
|                | ↑ TNF-α | ↑ TNF-α | ↑ TNF-α | ↑ TNF-α | ↑ TNF-α ND* | ↑ TNF-α |
|                | ↑ CXCL2 | ↑ CXCL2 | ↑ CXCL2 | No effect CXCL2 | No effect CXCL2 | ↑ CXCL2 |
|                | ↑ CCL2 | ↑ CCL2 | ↑ CCL2 | ↑ CCL2 | No effect CCL2 | ↑ CCL2 |

*ND: not detectable.

| PPAR-γ agonist | Pam3Cys | polyI:C | LPS | Flagellin | ssRNA | ODN |
|----------------|---------|---------|-----|-----------|-------|-----|
| 15d-PGJ2       | ↑ CXCL2 | ↑ CXCL2 | ↑ NO | ↑ NO | ↑ CXCL2 |

| PPAR-γ agonist | Pam3Cys | polyI:C | LPS | Flagellin | ssRNA | ODN |
|----------------|---------|---------|-----|-----------|-------|-----|
| Ciglitazone    | ↑ CXCL2 | ↑ CXCL2 | ↑ CXCL2 | ↑ CXCL2 | NR* | ↑ CXCL2 |
| Pioglitazone   | ↑ CXCL2 | ↑ CXCL2 | ↑ CXCL2 | ↑ CXCL2 |
| Troglitazone   | ↑ CCL2 | ↑ CCL2 | NR | ↑ CCL2 | NR | ↑ CCL2 |

*NR: not reported.
**Figure 1:** The natural PPAR-γ agonist 15d-PGJ2 is a potent inhibitor of microglial IL-12 p40 production in response to a vast array of TLR ligands. Primary microglia were plated at 2 × 10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ2 prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of 15d-PGJ2 on microglial viability were assessed using an MTT assay (b). Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ2 are noted with asterisks (**P < .001). Results presented are representative of two independent experiments.

**Figure 2:** The synthetic PPAR-γ agonist rosiglitazone attenuates IL-12 p40 production in response to TLR ligands in microglia. Primary microglia were plated at 2 × 10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of rosiglitazone prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of rosiglitazone on microglial viability were assessed using an MTT assay (b). Significant differences between microglia treated with TLR ligands alone versus TLR ligands + rosiglitazone are noted with asterisks (*P < .05). Results presented are representative of two independent experiments.
Another proinflammatory cytokine with potent effects on the blood-brain barrier as well as glial activation is TNF-α [42]. This cytokine is expressed at high levels in numerous CNS infectious diseases including bacterial meningitis, brain abscess, as well as viral infections [43–46]. In some cases, excessive TNF-α production during these infectious diseases has been implicated in contributing to bystander damage to surrounding host tissue [45, 46]. Therefore, strategies aimed at achieving optimized cytokine expression may prove beneficial for favorable disease outcomes. Of the PPAR-γ agonists tested, 15d-PGJ2 was found to exert the most global inhibition of TNF-α production during these infectious diseases including bacterial meningitis, brain abscess, as well as viral infections [43–46]. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of pioglitazone on microglial viability were assessed using an MTT assay (b). Significant differences between microglia treated with TLR ligands alone versus TLR ligands + pioglitazone are noted with asterisks (∗P < .05; ∗∗P < .001). Results presented are representative of two independent experiments.

3.2. **PPAR-γ agonists differentially affect chemokine release by microglia following TLR ligand treatment**

Chemokines are small molecular weight (8–14 kDa) chemo-tactic cytokines that are produced locally at sites of inflammation and establish a concentration gradient resulting in the active recruitment of target cell populations [47]. Chemokines are a structurally and functionally related family of proteins subdivided into four groups based on the relative position of conserved N-terminal cysteine residues [47–49]. In general, the chemokine subfamilies show similar, often overlapping specificity with regards to the movements of the target cell populations they orchestrate. One key chemokine involved in the recruitment of neutrophils into areas of inflammation, including the CNS, is CXCL2 (MIP-2) [50–52]. The effects of 15d-PGJ2 on microglial CXCL2 expression were complex and varied with each TLR ligand. Specifically, CXCL2 release was either enhanced (polyI:C, LPS, and ODN), reduced (Pam3Cys4), or remained unchanged (flagellin and ssRNA) (Figure 6). Increases in CXCL2 production were also observed following pioglitazone treatment in Pam3Cys- and ODN-stimulated microglia (Figure 7). The overall stimulatory activity of 15d-PGJ2 on CXCL2 production is in agreement with reports from other groups [53, 54].

Another chemokine that is associated with mononuclear cell infiltration during various CNS infections is
regulated by 15d-PGJ2 in response to diverse TLR ligands, γ3.3. Effects of PPAR-γ agonists on astrocytic proinflammatory mediator production in response to TLR ligands.

Astrocytes participate in CNS innate immune responses as evident by their ability to produce a wide array of inflammatory mediators in response to diverse stimuli [1, 4]. As already mentioned, these molecules can have dramatic consequences on CNS infection and tissue damage with net effects dictated by factors such as timing and duration of release. To determine the effects of PPAR-γ agonists on astrocyte responses to TLR ligands, we examined the production of two proinflammatory mediators produced by activated astrocytes, namely, NO and IL-12 p40. Both poly(I:C) and LPS were capable of reproducibly inducing NO expression in astrocytes as previously described [31, 57], which was attenuated by 15d-PGJ2 in a dose-dependent manner (Figure 9). Similar inhibitory effects on astrocytic NO release in response to poly(I:C) and LPS were observed with troglitazone and ciglitazone, whereas rosiglitazone and pioglitazone did not modulate NO production (data not shown). Of note was the fact that unlike microglia, which exhibited significant cell death in response to the highest dose of 15d-PGJ2 tested (i.e., 20 μM), astrocyte viability was not adversely affected by 15d-PGJ2 at any of the concentrations examined. This finding is in agreement with previous reports demonstrating that, in general, primary astrocytes are more refractory to the toxic effects of PPAR-γ agonists compared to primary microglia [23, 24, 27].

Similar to the findings obtained with microglia, 15d-PGJ2 was a universal and potent inhibitor of IL-12 p40 production by astrocytes in response to all TLR ligands tested

CCL2 (MCP-1), which targets monocyte and lymphocyte entry [52, 55, 56]. Unlike CXCL2, which was differentially regulated by 15d-PGJ2 in response to diverse TLR ligands, CCL2 production was uniformly and potently attenuated by this PPAR-γ agonist in response to the full array of TLR ligands tested (Figure 8). Similar to IL-12 p40 production, the synthetic TZDs demonstrated differential effects on CCL2 release from TLR ligand activated microglia. Specifically, rosiglitazone was fairly comprehensive in its ability to attenuate CCL2 production with significant reductions observed in response to Pam3Cys4, polyI:C, LPS, flagellin, and ODN, whereas the other TZDs tested (ciglitazone and pioglitazone) did not have much effect on CCL2 release in response to the majority of TLR ligands tested (data not shown). In summary, these results reveal that PPAR-γ agonists display a wide range of effects on chemokine production by microglia elicited by TLR ligands.

3.3. Effects of PPAR-γ agonists on astrocytic proinflammatory mediator production in response to TLR ligands

Primary microglia were plated at 2 x 10⁵ cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ2 prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon TNF-α levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ2 are noted with asterisks (*P < .05; **P < .001). Results presented are representative of two independent experiments.

As already mentioned, these molecules can have dramatic consequences on CNS infection and tissue damage with net effects dictated by factors such as timing and duration of release. To determine the effects of PPAR-γ agonists on astrocyte responses to TLR ligands, we examined the production of two proinflammatory mediators produced by activated astrocytes, namely, NO and IL-12 p40. Both poly(I:C) and LPS were capable of reproducibly inducing NO expression in astrocytes as previously described [31, 57], which was attenuated by 15d-PGJ2 in a dose-dependent manner (Figure 9). Similar inhibitory effects on astrocytic NO release in response to poly(I:C) and LPS were observed with troglitazone and ciglitazone, whereas rosiglitazone and pioglitazone did not modulate NO production (data not shown). Of note was the fact that unlike microglia, which exhibited significant cell death in response to the highest dose of 15d-PGJ2 tested (i.e., 20 μM), astrocyte viability was not adversely affected by 15d-PGJ2 at any of the concentrations examined. This finding is in agreement with previous reports demonstrating that, in general, primary astrocytes are more refractory to the toxic effects of PPAR-γ agonists compared to primary microglia [23, 24, 27].

Similar to the findings obtained with microglia, 15d-PGJ2 was a universal and potent inhibitor of IL-12 p40 production by astrocytes in response to all TLR ligands tested
3.4. **PPAR-γ agonists modulate chemokine production by astrocytes following TLR ligand exposure**

Although astrocytes are capable of releasing cytokines in response to diverse antigens, they are often considered the major source of chemokines during CNS inflammation [1, 4]. Similar to our recent report, 15d-PGJ$_2$ slightly augmented CXCL2 production by astrocytes in response to several TLR ligands examined, namely Pam3Cys4, PGN, and LPS (Figure 12 and data not shown) [24]. Similar increases in CXCL2 release following PPAR-γ agonist exposure have also been reported by others [53, 54]. In contrast, each synthetic TZD appeared to differentially regulate CXCL2 release. For example, ciglitazone inhibited CXCL2 expression in response to the entire battery of TLR ligands examined (Figure 13). Conversely, pioglitazone significantly augmented CXCL2 expression in response to polyI:C, flagellin, and ssRNA particularly at the highest dose examined (i.e., 100 μM; Figure 14), whereas rosiglitazone did not dramatically alter CXCL2 levels response to any of the TLR ligands tested in astrocytes (data not shown).

Similar to CXCL2, pioglitazone treatment increased CCL2 production in response to flagellin, and ssRNA primarily at the highest dose tested, whereas troglitazone led to significant reductions in CCL2 release following Pam3Cys4, polyI:C, flagellin, and ODN treatment (Figure 15). In general, ciglitazone and rosiglitazone had little effect on CCL2 production by astrocytes in response to the majority of TLR ligands tested (data not shown). These results indicate that despite their inclusion within the same family, specific PPAR-γ agonists differentially target proinflammatory genes in distinct manners.

4. **DISCUSSION**

In order for the CNS to respond to infectious insults, a rapid and efficient host immune response must be initiated and directed to expedite pathogen elimination. One means to achieve this goal is through the triggering of TLRs expressed on resident glia that signal proinflammatory mediator release in an attempt to quell infection [11, 12]. However, recent evidence also suggests that these normally protective immune responses can become dysregulated, culminating...
The finding that 15d-PGJ2 consistently led to agonists tested to modulate TLR-dependent glial activation in response to a wide range of TLR ligands. Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ2 prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, upon which CCL2 levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ2 are noted with asterisks (\* P < .05; \*\* P < .001). Results presented are representative of two independent experiments.

Our findings demonstrating the inhibitory effects of 15d-PGJ2 on TLR ligand-induced glial activation are similar to those observed following LPS stimulation. Specifically, 15d-PGJ2 has been shown to inhibit LPS-induced NO [27–29, 66], TNF-α [27, 28, 66], and IL-12 family member expression [26] in microglia and/or astrocytes. Importantly, this report has provided a more comprehensive analysis of the effects of PPAR-γ agonists on glial activation with the inclusion of a battery of TLR ligands that would be encountered during various CNS infectious diseases. Earlier studies have primarily focused on the immune modulatory effects of PPAR-γ agonists in response to the TLR4 ligand LPS, and therefore, this study is also novel from the perspective that numerous TLR ligands were evaluated.

Interestingly, both 15d-PGJ2 and pioglitazone were found to augment CXCL2 release by microglia and astrocytes in response to specific TLR ligands. In particular, 15d-PGJ2 enhanced CXCL2 production in response to LPS stimulation.
Figure 9: 15d-PGJ2 attenuates NO production by astrocytes in response to polyI:C and LPS stimulation. Primary astrocytes were plated at $1 \times 10^5$ cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ2 prior to the addition of polyI:C or LPS. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon NO levels were determined by the Griess reagent (a). The effects of 15d-PGJ2 on astrocyte viability were assessed using an MTT assay (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + 15d-PGJ2 are noted with asterisks ($^* P < .05$; $^{**} P < .001$). Results presented are representative of two independent experiments.

Figure 10: 15d-PGJ2 is a global inhibitor of astrocytic IL-12 p40 release following TLR ligand exposure. Primary astrocytes were plated at $1 \times 10^5$ cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ2 prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a) and (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + 15d-PGJ2 are noted with asterisks ($^* P < .05$; $^{**} P < .001$). Results presented are representative of two independent experiments.
Figure 11: The TZD pioglitazone inhibits astrocytic IL-12 p40 expression in response to diverse TLR ligands. Primary astrocytes were plated at $1 \times 10^5$ cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of pioglitazone on astrocyte viability were assessed using an MTT assay (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + pioglitazone are noted with asterisks ($^* P < .05; ^{**} P < .001$). Results presented are representative of two independent experiments.

Figure 12: CXCL2 release is augmented in astrocytes by 15d-PGJ$_2$ in response to distinct TLR ligands. Primary astrocytes were plated at $1 \times 10^5$ cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of Pam3Cys4 or LPS. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CXCL2 levels were determined by ELISA (a). The effects of 15d-PGJ$_2$ on astrocyte viability were assessed using an MTT assay (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks ($^* P < .05; ^{**} P < .001$). Results presented are representative of two independent experiments.
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Figure 13: Ciglitazone attenuates astrocytic CXCL2 expression in response to several TLR ligands. Primary astrocytes were plated at 1 × 10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of ciglitazone prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CXCL2 levels were determined by ELISA (a). The effects of ciglitazone on astrocyte viability were assessed using an MTT assay (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + ciglitazone are noted with asterisks (*) P < .05; **P < .001). Results presented are representative of two independent experiments.

Figure 14: Pioglitazone enhances CXCL2 release by astrocytes following TLR ligand exposure. Primary astrocytes were plated at 1 × 10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone prior to the addition of polyIC, flagellin, or ssRNA. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CXCL2 levels were determined by ELISA. Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + pioglitazone are noted with asterisks (*) P < .05; **P < .001). Results presented are representative of two independent experiments.

in both microglia and astrocytes, in agreement with previous reports [53, 54]. In addition, recent results from our laboratory have revealed that CXCL2 release was slightly increased by 15d-PGJ2 and downregulated by ciglitazone in response to the gram-positive pathogen S. aureus in primary astrocytes [24], in corroboration with the current report.

Elevated levels of endogenous 15d-PGJ2 have been associated with the resolution of inflammation in vivo, suggesting that it functions as negative feedback regulator of inflammatory responses [67, 68]. This study demonstrates that 15d-PGJ2 is an effective and selective inhibitor of glial activation in response to TLR ligands, suggesting that it may be capable of modulating chronic microglial and astrocyte responses during the course of CNS infectious diseases. Evidence to support this concept is provided by our findings that 15d-PGJ2 effectively inhibited IL-12 p40 and CCL2 expression in microglia and astrocytes, two molecules with important functions in the differentiation of CD4+ Th1 cells and monocyte and T cell influx into the infected CNS [52, 55, 56, 69, 70]. It is possible that the downregulation of these mediators in microglia and astrocytes following 15d-PGJ2 treatment results in the failure to recruit and/or stimulate Ag-specific T cells in the CNS parenchyma. Therefore, preventing chronic microglial activation by 15d-PGJ2 or synthetic PPAR-γ agonists may help to resolve inflammation earlier, resulting in less damage to surrounding normal brain parenchyma.

In summary, these studies demonstrate that not all PPAR-γ agonists are created equal in terms of their ability to modulate proinflammatory mediator release by activated microglia and astrocytes. Specifically, differences were
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addition, recent studies by other groups have revealed beneficial effects of PPAR-γ agonists in cerebral innate immunity [71].

mental brain abscess model as revealed by accelerated abscess encapsulation and reduction in bacterial burdens [71]. In addition, recent studies by other groups have revealed beneficial effects of PPAR-γ agonists in other infectious disease paradigms [72–76]. Therefore, the current study can be used as a guide to facilitate the selection of PPAR-γ agonists as candidates for intervention during CNS infectious diseases.

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