The Journal of Immunology

The Synthetic Peroxisome Proliferator-Activated Receptor- γ Agonist Ciglitazone Attenuates Neuroinflammation and Accelerates Encapsulation in Bacterial Brain Abscesses

Tammy Kielian, Mohsin Md. Syed, Shuliang Liu, Nirmal K. Phulwani, Napoleon Phillips, Gail Wagoner, Paul D. Drew and Nilufer Esen

J Immunol 2008; 180:5004-5016; ;
doi: 10.4049/jimmunol.180.7.5004
http://www.jimmunol.org/content/180/7/5004

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

The Synthetic Peroxisome Proliferator-Activated Receptor-γ Agonist Ciglitazone Attenuates Neuroinflammation and Accelerates Encapsulation in Bacterial Brain Abscesses[1]

Tammy Kielian,[2] Mohsin Md. Syed, Shuliang Liu, Nirmal K. Phulwani, Napoleon Phillips, Gail Wagoner, Paul D. Drew, and Nilufer Esen

Brain abscesses result from a pyogenic parenchymal infection commonly initiated by Gram-positive bacteria such as *Staphylococcus aureus*. Although the host immune response elicited following infection is essential for effective bacterial containment, this response also contributes to the significant loss of brain parenchyma by necrosis that may be reduced by modulating the inflammatory response. Ciglitazone, a PPAR-γ agonist with anti-inflammatory properties, was evaluated for its ability to influence the course of brain abscess development when treatment was initiated 3 days following infection. Interestingly, abscess-associated bacterial burdens were significantly lower following ciglitazone administration, which could be explained, in part, by the finding that ciglitazone enhanced *S. aureus* phagocytosis by microglia. In addition, ciglitazone attenuated the expression of select inflammatory mediators during brain abscess development including inducible NO synthase, TNF-α, IL-1β, CXCL2, and CCL3. Unexpectedly, ciglitazone also accelerated brain abscess encapsulation, which was typified by the heightened expression of fibronectin and α-smooth muscle actin-positive myofibroblasts. Collectively, through its ability to attenuate excessive inflammation and accelerate abscess encapsulation, ciglitazone may effectively sequester brain abscesses and limit bacterial dissemination. ***The Journal of Immunology,*** **2008, 180: 5004–5016.**

Pyogenic bacterial infections in the CNS parenchyma often lead to the development of abscesses. Common pathogens associated with brain abscesses in humans include streptococcal strains and *Staphylococcus aureus* (1, 2). Because of its frequent association with brain abscesses, our laboratory has developed an experimental brain abscess model in the mouse using *S. aureus* (3–5). This model accurately recapitulates the sequence of events and pathological sequelae that occur during brain abscess progression in humans, providing an excellent platform to investigate potential therapeutic interventions to minimize the extensive damage to the brain parenchyma that is characteristic of infection.

Brain abscesses evolve through a series of well-defined stages (6, 7). The initial phase is acute cerebritis, which is typified by extensive edema and the significant influx of neutrophils into the infected parenchyma. This edematous response occurs within the first few hours after infection and continues until ~3 days following pathogen exposure, while neutrophil influx persists throughout the entire course of disease. Microglial and astrocyte activation can also be visualized along the interface between the infected and neighboring tissue during the acute cerebritis phase. The intermediate stage of brain abscess development occurs around days 3–7 postinfection and is associated with a large accumulation of macrophages along the margin of necrotic tissue and surrounding uninfected brain parenchyma. The final stage, also referred to as late cerebritis, spans from day 7 onward and is associated with the formation of a fibrotic wall surrounding the abscess in an attempt to protect neighboring parenchyma from infection. This fibrotic wall is associated with the appearance of myofibroblasts and the deposition of extracellular matrix components including fibronectin and collagen (7). A significant vascular response is also observed along the developing wall in conjunction with the clustering of activated macrophages/microglia. Interestingly, over time the abscess cavity regresses in size, which may be attributed to the contractile nature of myofibroblasts (8, 9). The presence of bacteria and the vigorous inflammatory cell infiltrate that ensues to contain the infection lead to liquifactive necrosis of the affected brain parenchyma and exudate formation. As the infection resolves, a significant portion of brain tissue has been permanently lost and is typically too expansive for a regenerative response. As a consequence, patients suffering from brain abscesses often experience permanent neurological deficits that are lesion site-dependent including seizures, cognitive decline, or hemiparesis (10). Therefore, therapies aimed at reducing the extent of parenchymal damage following infection could lead to significant improvements in clinical outcomes and quality of life for patients recovering from brain abscesses (11).

A group of compounds with reported anti-inflammatory effects in several models of inflammation, including the CNS, are ligands

Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Received for publication September 11, 2007. Accepted for publication January 23, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by 2RO1 NS40730 from the National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH) (to T.K.) and P30 NS047546 from the NINDS-supported Core Facility at the University of Arkansas for Medical Sciences. Support for the Digital and Confocal Microscopy Laboratory at the University of Arkansas for Medical Sciences is provided by Grant P20 RR6460 from NIH/IDeA Network of Biomedical Research Excellence and Grant S10 RR19395 from NIH/National Center for Research Resources.

[2] Address correspondence and reprint requests to Dr. Tammy Kielian, Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, 4301 West Markham Street, Slot 846, Little Rock, AR 72205. E-mail address: KielianTammyL@uams.edu

that interact with peroxisome proliferator-activated receptor-γ (PPAR-γ)[3] (12, 13). 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 (13, 14). A wide array of both natural and synthetic agonists for PPAR-γ have been identified including the naturally occurring prostaglandin metabolite 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), thiazoladinediones a group of synthetic PPAR-γ agonists used for the treatment of diabetes, polyunsaturated fatty acids, and certain high-affinity 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 their ability to impact the course of several neurodegenerative diseases (12, 15–17). Pertinent to brain abscesses, our group has recently shown that the natural and synthetic PPAR-γ ligands 15d-PGJ_2 and ciglitazone, respectively, are effective inhibitors of *S. aureus*-dependent activation of both microglia and astrocytes (18, 19).

Based on the capacity of PPAR-γ agonists to attenuate inflammation, the synthetic thiazoladinedione ciglitazone was evaluated for its ability to modulate the course of brain abscess development. Ciglitazone exerted anti-inflammatory effects in a post-treatment paradigm in which animals received the drug beginning at 3 days following brain abscess induction, as typified by the attenuated production of numerous proinflammatory mediators, including inducible NO synthase (iNOS), TNF-α, IL-1β, CXCL2 (MIP-2), and CCL3 (MIP-1α). In addition, the degree of microglial/macrophage activation associated with brain abscesses of ciglitazone-treated mice was reduced compared with vehicle control animals, which was in agreement with the ability of the drug to inhibit microglial proinflammatory mediator expression when administered either prior or subsequent to *S. aureus* exposure in vitro. Brain abscess bacterial burdens were significantly lower following ciglitazone administration, which could be explained, in part, by the ability of ciglitazone to augment *S. aureus* phagocytosis by microglia. Unexpectedly, ciglitazone accelerated brain abscess encapsulation, which was typified by the heightened expression of fibronectin and α-smooth muscle actin-positive myofibroblasts. Collectively, these results suggest that ciglitazone may represent a potential adjunctive therapy with antibiotics for the clinical management of brain abscesses through its ability to accelerate abscess encapsulation and potentially restrict bacterial dissemination.

Materials and Methods

Bacteria and reagents

The *S. aureus* strain RN6390 was used as previously described (20, 21). The synthetic PPAR-γ agonist ciglitazone was purchased from Cayman Chemical and recombinant mouse IFN-γ was obtained from BD Biosciences.

Generation of experimental brain abscesses

Brain abscesses were induced in 6- to 8-wk-old C57BL/6 mice using a stereotaxic approach as previously described (3, 4). For all studies, an equal number of age-matched males and females were used as previous work has established that both genders exhibit qualitatively similar inflammatory profiles following bacterial challenge (3, 4, 20, 21). The animal use protocol has been approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee and is in accord with the National Institutes of Health guidelines for the use of rodents.

For evaluating the potential therapeutic effects of ciglitazone during brain abscess development, mice were subjected to a post-treatment paradigm in which the drug was examined for its ability to modulate ongoing CNS inflammation. For these studies, ciglitazone was administered 3 days following *S. aureus* infection, a time point at which bacterial burdens have nearly peaked (21, 22). After this initial treatment, injections continued daily until the termination of the study. Ciglitazone was initially examined at three concentrations (2, 10, and 50 mg/kg/day); however, in subsequent studies one dose was selected (50 mg/kg/day), which was determined to exert optimal effects on the ensuing host CNS antibacterial response. For all experiments, one group of animals received treatment with the highest concentration of ciglitazone alone to assess any effects of the drug independently of *S. aureus* infection. Animals receiving ciglitazone alone did not demonstrate any overt adverse effects to the drug (i.e., no significant alterations in body weight). The doses of ciglitazone tested are representative of PPAR-γ agonist concentrations used in previously published reports of disparate CNS neurodegenerative models (23–25).

Simultaneous collection of RNA, protein, and quantitation of viable bacteria from brain abscesses

Brain abscesses were visualized by the stab wound created during injections, sectioned within 2–3 mm on all sides, and homogenized in PBS supplemented with protease and RNase inhibitors. Subsequently, RNA, protein, and bacterial titers were obtained as previously described (4).

Quantitation of brain abscess area

Brain abscess sizes in ciglitazone- and vehicle-treated mice were calculated as previously described (26).

Immunofluorescence staining and confocal microscopy

The effects of ciglitazone on microglial/macrophage activation and myofibroblast accumulation during brain abscess development were evaluated by immunofluorescence staining using Iba-1 and α-smooth muscle actin, respectively. In addition, PPAR-γ localization and fibronectin deposition in brain abscesses was also examined. Brain abscess tissues were collected at the indicated time points following ciglitazone treatment, and 10-μm cryostat sections were mounted onto SuperFrost Plus slides (Fisher Scientific), air dried, and stored at −80°C until use. For the majority of experiments presented, fresh frozen tissues were analyzed; however, to detect Iba-1 immunoreactivity, tissues fixed in 4% paraformaldehyde were used because strong staining with this Ab was not detected in fresh frozen sections. Regardless of the fixation procedure, staining was initiated by equilibrating slides at room temperature for 15 min followed by incubation in ice-cold methanol. Following numerous rinses in PBS, tissues were incubated with PBS/10% normal donkey serum to minimize nonspecific staining. Brain abscess tissue was reacted with either goat anti-mouse Iba-1 (Abcam), rabbit anti-mouse fibronectin (Affinity Bioreagents), rabbit anti-mouse α-smooth muscle actin (Epitomics), or rabbit anti-mouse PPAR-γ (Cayman Chemicals) Abs overnight at 4°C in a humidified chamber. Following numerous rinses in PBS, tissues were incubated with either donkey anti-goat IgG-FITC (for Iba-1) or biotinylated donkey anti-rabbit IgG (for fibronectin, α-smooth muscle actin, and PPAR-γ) Abs for 1 h at room temperature. All secondary Abs were purchased from Jackson ImmunoResearch Laboratories. Fibronectin, α-smooth muscle actin, and PPAR-γ expression were visualized by the addition of a streptavidin-Alexa Fluor 568 conjugate (Molecular Probes). Upon completion of the staining protocol, slides were cover slipped using the Prolong anti-fade reagent (Molecular Probes) and sealed using nail polish. Slides were imaged using a LSM 510 laser scanning confocal microscope (Carl Zeiss Microimaging). Specific staining of Abs was confirmed by absence of fluorescence signal following incubation of brain abscess tissues with secondary Abs alone (data not shown).

Western blotting

Determination of iNOS expression in brain abscesses of ciglitazone- and vehicle-treated mice was performed by Western blotting with a polyclonal Ab against iNOS (Santa Cruz Biotechnology) as previously described (4).

ELISA

Murine IL-1β and CXCL2 were quantified in brain abscess homogenates or primary microglial supernatants using commercial ELISA kits (DuoSet; R&D Systems). Results were normalized to the amount of total protein extracted from tissues to correct for differences in sampling size as previously described (3, 22).

[3] Abbreviations used in this paper: PPAR-γ, peroxisome proliferator-activated receptor-γ; iNOS, inducible NO synthase; PGN, peptidoglycan; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; EAE, experimental autoimmune encephalomyelitis.

Multianalyte microbead array to detect proinflammatory mediator expression

To expand the analysis of inflammatory mediators modulated by ciglitazone during brain abscess development, a mouse 20-plex cytokine microbead array system was used according to the manufacturer's instructions as previously described (catalog no. LMC0006; BioSource International) (5). Results were analyzed using a Bio-Plex Workstation (Bio-Rad) and adjusted based on the amount of total protein extracted from abscess tissues for normalization. The level of sensitivity for each microbead cytokine standard curve ranged from 1 to 35 pg/ml.

Preparation of primary microglia

Primary microglia were isolated from neonatal C57BL/6 mice (postnatal day 2–4) as previously described (27). To investigate the effects of ciglitazone on microglial activation, primary microglia were seeded into 96-well plates at 2×10^5 cells/well and incubated overnight. The following day, cells were treated with various doses of ciglitazone for 1 h before stimulation with either heat-inactivated *S. aureus* (10^7 CFU; strain RN6390) or 10 μg/ml peptidoglycan (PGN; InvivoGen). Cell-conditioned supernatants were collected at 24 h following bacterial exposure, and IL-1β and CXCL2 production were evaluated by ELISA. In experiments designed to examine whether ciglitazone was capable of modulating preexisting microglial activation, cells were exposed to ciglitazone 6 h following bacterial stimulation, and proinflammatory mediator expression was quantitated by ELISA. The doses of bacterial stimuli used were based on our previous studies that established optimal cytokine responses without any evidence of toxicity (19, 28).

Nitrite assay

Nitrite, a stable end product of resulting from the reaction of NO with molecular oxygen, was used to quantitate NO levels in microglial-conditioned supernatants as previously described (19).

Phagocytosis assay

The effect of ciglitazone on microglial phagocytosis of *S. aureus* was performed as previously described (29). Briefly, C57BL/6 primary microglia were seeded onto 12 mm coverslips (2×10^5 cells/coverslip) placed in 24-well plates and incubated overnight. The following day, cells were pretreated with either medium alone or 50 μM ciglitazone for 24 h to allow sufficient time for PPAR-γ response element-driven changes in gene expression to be manifested at the protein level. After the 24-h period, microglia were incubated with a heat-killed *S. aureus* isolate that constitutively expresses GFP, provided by Dr. A. Cheung (Dartmouth Medical School, Hanover, NH) for 2 h. Cells were washed extensively with PBS and incubated with 0.05% crystal violet in 0.15 M NaCl for 45 s to quench any fluorescence signal from residual extracellular bacteria. Coverslips were mounted onto glass slides using the Prolong anti-fade reagent (Molecular Probes), sealed using nail polish, and imaged using an LSM 510 laser scanning confocal microscope (Carl Zeiss Microimaging).

Cell viability assays

The effects of ciglitazone on microglial viability were evaluated using a standard assay based upon the mitochondrial conversion of MTT into formazan crystals as previously described (18).

Statistics

Significant differences between experimental groups were determined using the unpaired Student's *t* test at the 95% confidence interval with Sigma Stat (SPSS Science). This analysis was determined to be most appropriate because, although we are evaluating changes in proinflammatory mediator expression over time, repeated measurements are not made on the same animal (mice are sacrificed to collect abscess homogenates at each time point), precluding ANOVA and posthoc analysis of the data.

In this study, we performed a minimum of two independent replicates of each in vivo experiment to confirm the results obtained. The reporting of our results as representative of *x* number of independent experiments was required because it is difficult to achieve identical bacterial burdens in mice between independent brain abscess studies with distinct bacterial preparations. As a result, the absolute concentrations of the various proinflammatory mediators detected within brain abscesses of ciglitazone- and vehicle-treated mice differed between individual experiments; however, the trends were consistent. This required us to report results from a single experiment in which at least four to six mice per time point per group were analyzed and statistical analysis conducted.

Results

The synthetic PPAR-γ agonist ciglitazone is effective at reducing inflammation when administered subsequent to bacterial infection

Previous studies in the brain abscess model have demonstrated that the proinflammatory mediators IL-1, TNF-α, and CXCL2 play an important role in the host immune response to infection (3, 21, 30). However, the sustained production of these same mediators can also negatively impact cellular survival as demonstrated by others (31–36). Based on the fact that proinflammatory mediator expression continues during the later stages of brain abscess in the face of relatively few surviving bacteria (22), we propose that this persistent cytokine response is deleterious, possibly confounding disease through the exaggerated destruction of normal brain parenchyma surrounding the abscess. Therefore, the synthetic PPAR-γ agonist ciglitazone was tested for its ability to modulate the ensuing host inflammatory response for optimal bacterial killing with minimal bystander destruction to brain tissue.

We first sought to determine whether ciglitazone could affect established inflammation during brain abscess evolution because this strategy represents a plausible clinical approach to modulate infection. In these studies, mice received single daily doses of ciglitazone beginning at day 3 post-infection, a time point at which bacterial burdens have nearly peaked (21, 22). Animals were sacrificed at 1 and 3 days following ciglitazone treatment for analysis, which corresponded to days 4 and 6 post-infection, respectively. Ciglitazone significantly attenuated the expression of several proinflammatory mediators including TNF-α, IL-1β, CXCL2, and CCL3 (Fig. 1).

NO is a reactive nitrogen intermediate with potent antimicrobial and immune modulatory effects. It is produced by any one of three enzyme isoforms termed NO synthases. Expression of iNOS is stimulated in glia and mononuclear phagocytes by a variety of inflammatory cytokines and bacterial products, including LPS, *S. aureus*, and PGN (27, 37). Quantitation of iNOS levels is routinely used to reflect NO production in vivo because the free radical itself is highly unstable and difficult to measure accurately in tissue extracts. To determine whether iNOS expression in brain abscesses was modulated by ciglitazone, we compared iNOS levels in lesions from ciglitazone- and vehicle-treated mice. Similar to TNF-α, IL-1β, CXCL2, and CCL3, iNOS expression was markedly reduced in brain abscesses of ciglitazone-treated animals at all time points examined (Fig. 2). Interestingly, not all molecules evaluated were affected by ciglitazone treatment as evident by the inability of the drug to modulate IL-12 p70, IL-23 p19, KC, or IL-10 expression (data not shown). Another important finding was that ciglitazone was only capable of partially attenuating proinflammatory mediator production (Figs. 1 and 2), because total blockade of these responses would likely lead to adverse effects from an inability to completely clear bacteria from the brain parenchyma.

Surprisingly, despite the fact that the expression of several proinflammatory mediators considered to be key for effective antibacterial immunity were reduced by ciglitazone administration, bacterial burdens in brain abscesses of ciglitazone treated mice were significantly lower compared with vehicle-treated animals (Fig. 3). This finding may be explained by the reported ability of ciglitazone to influence key components of the phagocytic pathway including augmenting the scavenger receptor CD36 as well as catalase expression (38, 39) because this PPAR-γ agonist does not possess any direct antimicrobial activity.

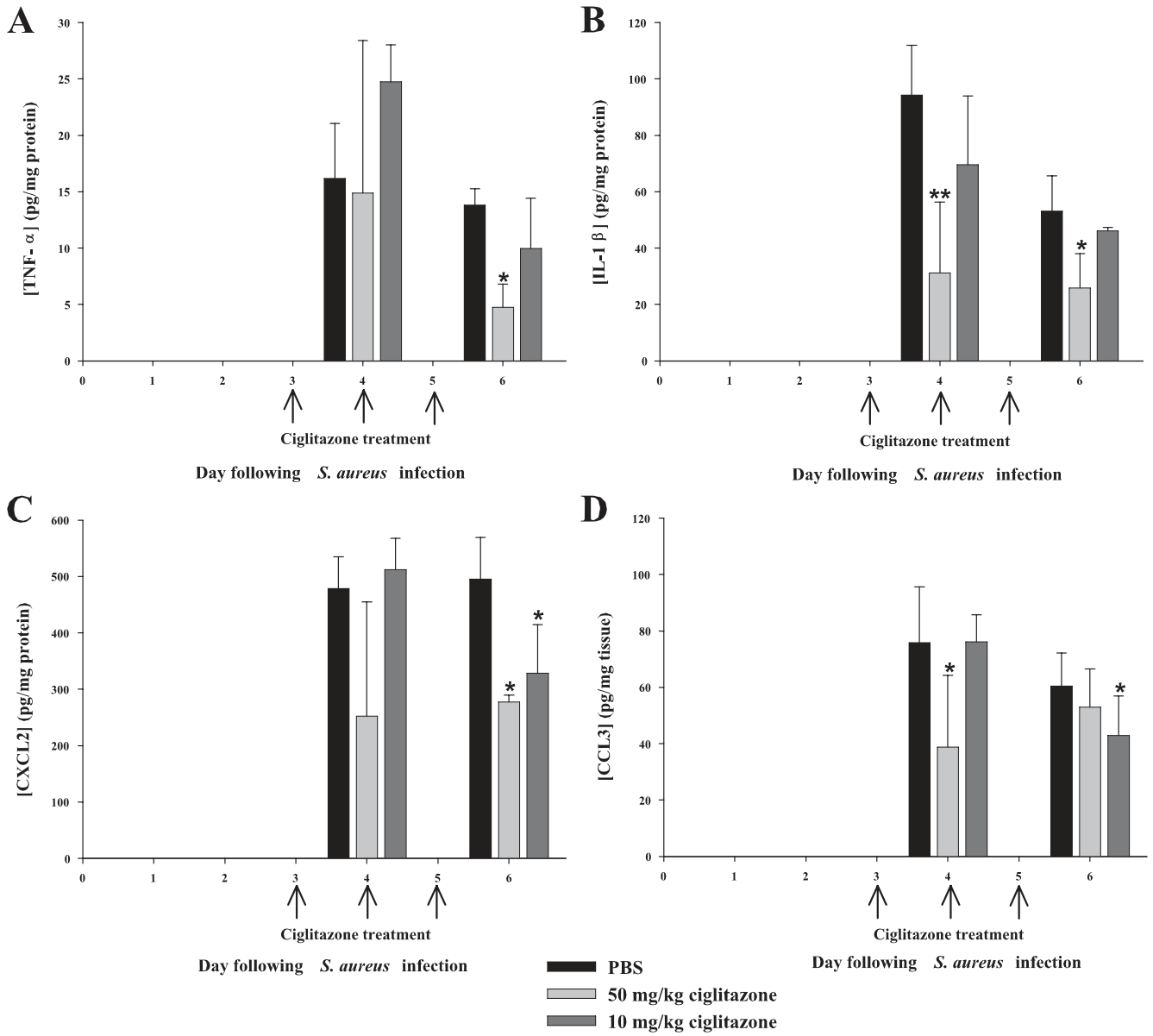

FIGURE 1. Delayed ciglitazone administration is capable of attenuating proinflammatory mediator expression in brain abscesses. Mice ($n = 4–6$ per group) received i.p. injections of PBS or ciglitazone at the indicated concentrations (10 or 50 mg/kg/day) beginning at 3 days following an intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at either 1 or 3 days after ciglitazone treatment (corresponding to days 4 and 6 post-infection), whereupon TNF-α (*A*), IL-1β (*B*), CXCL2 (*C*), and CCL3 (*D*) expression were evaluated by ELISA or multiplex bead arrays and results normalized to the amount of total protein recovered from abscesses to correct for differences in tissue sampling size. *, $p < 0.05$; **, $p < 0.001$ represent significant differences between infected mice treated with vehicle (PBS) vs ciglitazone. Results are representative of two independent experiments.

Ciglitazone administration accelerates brain abscess encapsulation, fibronectin deposition, and myofibroblast accumulation

Brain abscesses progress through a series of well-defined histological changes with a rather predictable time course (6, 7). Therefore, we were interested in determining whether ciglitazone exerted any effects on the nature of abscess evolution. Unexpectedly, administration of ciglitazone beginning at day 3 postinfection led to a heightened fibrotic reaction typified by accelerated abscess encapsulation (Fig. 4). In addition, fibronectin deposition was significantly augmented by ciglitazone along the abscess margins in the immediate vicinity of the developing wall (Fig. 5). In contrast, vehicle-treated animals demonstrated a less extensive network of fibronectin-positive cells (Fig. 5).

Another hallmark of the brain abscess wall is the appearance of myofibroblasts characterized by their expression of α-smooth muscle actin (40, 41). Within the normal CNS, α-smooth muscle actin is restricted to arteries/arterioles and the meninges. However, α-smooth muscle actin expressing myofibroblasts have been reported to organize along the developing brain abscess wall around days 9–10 following infection (7). To determine whether ciglitazone led to the accelerated appearance of myofibroblasts, immunofluorescence staining was performed. Similar to fibronectin deposition, α-smooth muscle actin expression was dramatically increased in ciglitazone-treated animals compared with controls (Fig. 6). In addition, α-smooth muscle actin myofibroblasts were more highly ordered following ciglitazone exposure, forming a compact network compared with vehicle-treated animals where

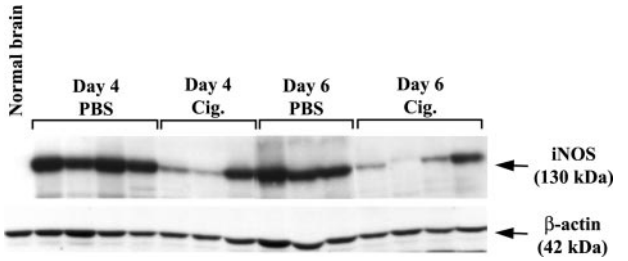

FIGURE 2. Ciglitazone attenuates iNOS expression in evolving brain abscesses. Mice received i.p. injections of PBS or 50 mg/kg ciglitazone beginning at 3 days following an intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at either 1 or 3 days after ciglitazone treatment (corresponding to days 4 and 6 post-infection), whereupon iNOS expression was evaluated by Western blotting (40 μg total protein/sample). Blots were stripped and reprobed with an Ab specific for β-actin to verify uniformity in gel loading. Results represent $n = 3$–4 individual animals per treatment group (with the exception of normal brain) and are representative of two independent experiments.

cells exhibited a sparse and ill-defined organization. The relative immaturity of the abscess wall in infected vehicle-treated mice at these early time points is expected given the time course of brain abscess evolution. Despite the ability of ciglitazone to enhance brain abscess encapsulation and fibrosis, lesion sizes were not significantly different between ciglitazone-treated and control animals at the time points examined (data not shown). Collectively, the coordinated increase in fibronectin and α-smooth muscle expression along the abscess margins by ciglitazone is indicative of accelerated myofibroblast organization and suggests that this PPAR-γ agonist prematurely redirects the host fibrotic response.

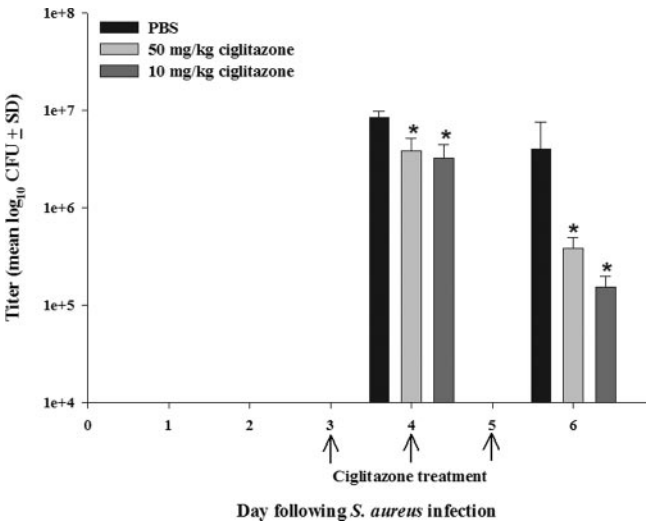

FIGURE 3. Ciglitazone significantly reduces bacterial burdens during brain abscess development. Mice ($n = 4$–6 per group) received i.p. injections of PBS or ciglitazone (10 or 50 mg/kg/day) beginning at 3 days following an intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at either 1 or 3 days after ciglitazone treatment (corresponding to days 4 and 6 post-infection) for quantitation of viable bacteria within abscesses. *, $p < 0.05$ reflects significant differences between infected PBS- and ciglitazone-treated animals. Results are representative of two independent experiments.

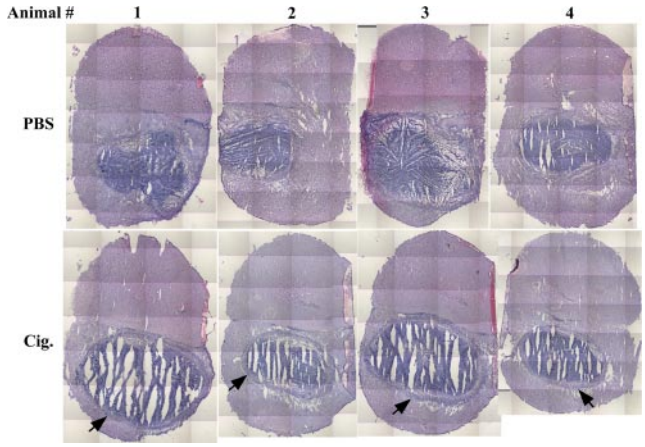

FIGURE 4. Ciglitazone leads to accelerated brain abscess encapsulation. Mice ($n = 4$–6 mice per group) received i.p. injections of PBS or ciglitazone (50 mg/kg/day) beginning at 3 days following an intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at day 3 following ciglitazone treatment (corresponding to day 6 post-infection), whereupon brain tissues were flash frozen on dry ice for subsequent cryostat sectioning. Serial sections were prepared throughout the entire abscess to ensure that the maximal cross-sectional area was identified and H&E staining performed to demarcate the histological appearance of lesions. A series of microscopic images at magnification ×4 were assembled for each individual vehicle- or ciglitazone-treated animal (lesions from $n = 4$ individual animals are presented) to demonstrate the extent of abscess formation. The fibrotic wall (along arrows) is more highly organized and extensive in ciglitazone-treated mice compared with controls (PBS). Results are representative of three independent experiments.

PPAR-γ immunoreactivity is localized along the developing brain abscess wall

Our results thus far have demonstrated that ciglitazone accelerates brain abscess encapsulation and formation of the fibrotic

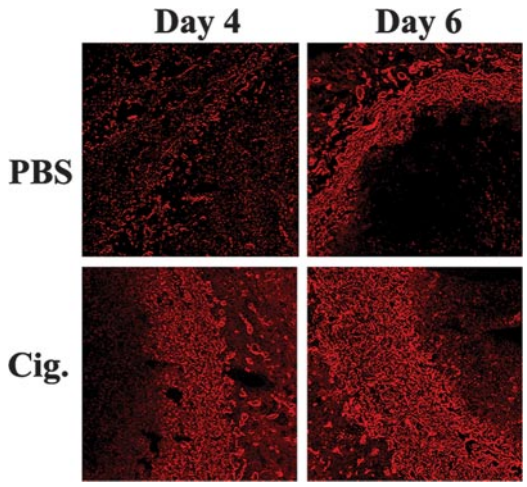

FIGURE 5. Fibronectin deposition along the developing brain abscess wall is enhanced by ciglitazone. Mice ($n = 5$–7 per group) received i.p. injections of PBS or ciglitazone (50 mg/kg/day) beginning at 3 days following an intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at either 1 or 3 days after ciglitazone treatment (corresponding to days 4 and 6 post-infection), whereupon brain tissues were flash frozen on dry ice for subsequent cryostat sectioning. Serial 10-μm thick sections were prepared throughout the entire abscess, subjected to immunofluorescence staining for fibronectin (red), and imaged by confocal microscopy at a magnification ×10. Fibronectin immunoreactivity is shown along the peri-abscess area in the vicinity of the developing wall, where the dark areas represent necrotic regions. Results are representative of three independent experiments.

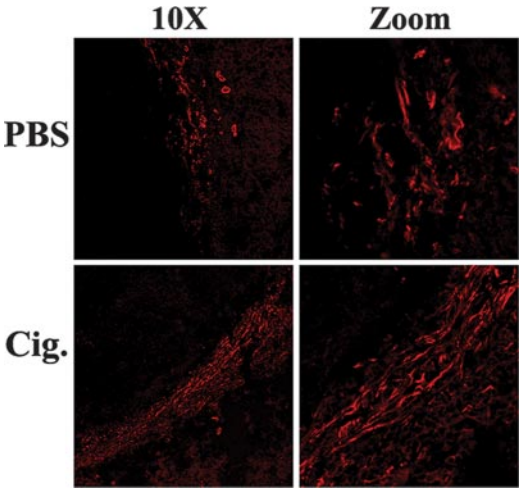

FIGURE 6. Ciglitazone accelerates the appearance and organization of α-smooth muscle actin-positive myofibroblasts. Mice (n = 5–7 per group) received i.p. injections of PBS or ciglitazone (50 mg/kg/day) beginning at 3 days following an intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at 3 days following ciglitazone treatment (corresponding to day 6 post-infection), whereupon brain tissues were flash frozen on dry ice for subsequent cryostat sectioning. Serial 10-μm thick sections were prepared throughout the entire abscess, subjected to immunofluorescence staining for α-smooth muscle actin (red), and imaged by confocal microscopy. α-smooth muscle actin immunoreactivity is shown along the peri-abscess area in the vicinity of the developing wall, where the dark areas represent necrotic regions. Enlarged zoom image represents a 63X oil immersion confocal scan. Results are representative of two independent experiments.

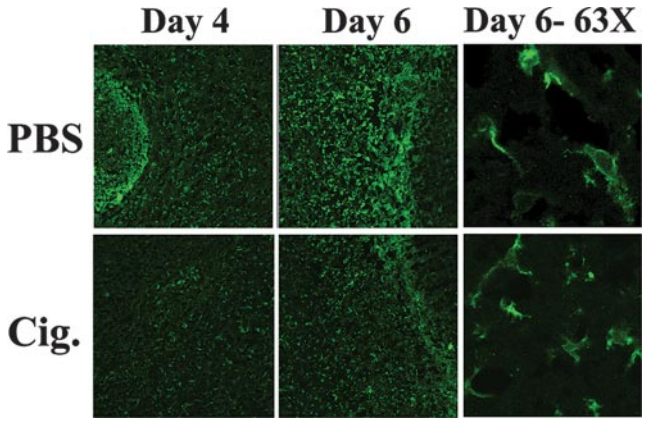

FIGURE 8. Microglia/macrophage activation within the peri-abscess tissue is reduced by ciglitazone. Mice (n = 5–7 per group) received i.p. injections of PBS or ciglitazone (50 mg/kg/day) beginning at 3 days following intracerebral infection with *S. aureus*, with treatment continuing daily until termination of the experiment. Animals were sacrificed at either 1 or 3 days after ciglitazone treatment (corresponding to days 4 and 6 post-infection), whereupon animals were perfused with 4% paraformaldehyde and fixed tissues were processed for cryostat sectioning. Serial 10-μm thick sections were prepared throughout the entire abscess, subjected to immunofluorescence staining for the microglial/macrophage marker Iba-1 (green), and imaged by confocal microscopy. Iba-1 immunoreactivity is shown along the peri-abscess area in the vicinity of the developing wall and extending into the surrounding parenchyma. *Left* and *middle panels* are at a magnification of $\times$10. High power images were taken to better visualize alterations in cellular morphology. *Right panels* use a 63X oil immersion confocal scan. Results are representative of one experiment evaluating at least n = 5–6 individual animals per group.

wall, which likely serves to limit bacterial dissemination. However, co-localizing the receptor for ciglitazone's action, PPAR-γ, to the developing brain abscess wall would further strengthen the region-dependent effects of this compound. Therefore, immunofluorescence staining was performed to delineate PPAR-γ expression in the infected brain parenchyma. Importantly, PPAR-γ immunoreactivity was primarily associated along the developing abscess wall (Fig. 7). We did not detect any significant differences in the frequency or intensity of PPAR-γ staining in brain abscesses of ciglitazone- or vehicle-treated mice, suggesting that ligand treatment does not significantly modulate PPAR-γ expression during infection (data not shown). Demonstration of robust PPAR-γ reactivity along the developing abscess wall provides strong evidence to suggest that this region is highly sensitive to the effects of PPAR-γ ligands such as ciglitazone.

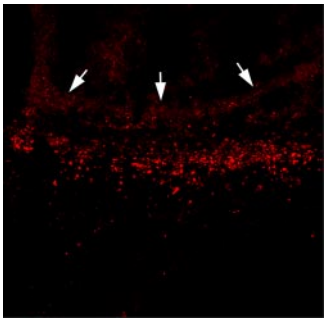

FIGURE 7. PPAR-γ immunoreactivity is localized along the developing brain abscess wall. Serial 10-μm thick sections were prepared throughout brain abscesses collected at day 6 post-infection, subjected to immunofluorescence staining for PPAR-γ expression (red), and imaged by confocal microscopy at a magnification of $\times$10. PPAR-γ immunoreactivity was detected within the peri-abscess tissue, where the developing abscess wall is demarcated (arrows). Results are representative of two independent experiments using a total of n = 10–12 individual mice.

Effects of ciglitazone on microglial/macrophage activation

In addition to examining changes associated with the developing brain abscess wall, we also investigated whether ciglitazone treatment influenced microglial/macrophage activation in vivo because previous studies from our laboratory have demonstrated that PPAR-γ agonists are potent inhibitors of *S. aureus*-dependent microglial activation (18). Examination of microglial/macrophage staining with Iba-1 revealed a progressive increase in staining over time, both along the peri-abscess margin and extending into the surrounding parenchyma, which is in agreement with the temporal activation and accumulation of these cells (7, 22). Interestingly, microglial/macrophage activation was attenuated by ciglitazone as revealed by a reduction in Iba-1 staining in the parenchyma neighboring the abscess, which was most prominent at 3 days following ciglitazone treatment (i.e., day 6 post-infection) (Fig. 8). In addition, higher magnification revealed that the morphology of Iba-1-positive cells was more ramified in brain abscesses of ciglitazone-treated mice suggestive of a less reactive state. In contrast, microglia/macrophages in vehicle-treated animals appeared more rounded and assumed a highly reactive phenotype (Fig. 8, image magnification with a 63X oil immersion confocal scanner). This finding is in agreement with the ability of ciglitazone to partially inhibit *S. aureus*-dependent microglial activation in vitro (Figs. 9 and 10).

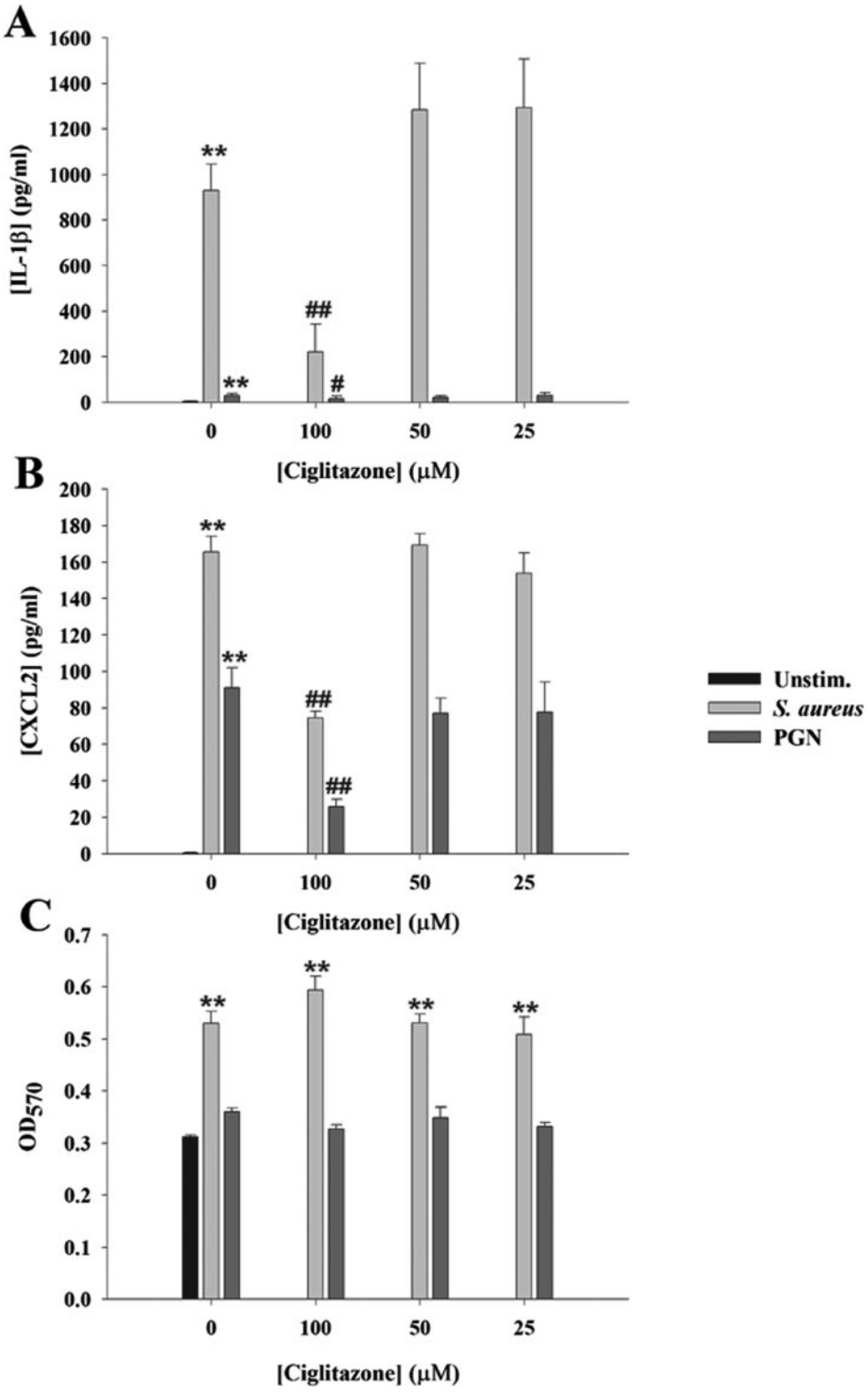

FIGURE 9. Ciglitazone attenuates *S. aureus*- and PGN-induced proinflammatory mediator expression in microglia. Primary microglia were seeded in 96-well plates at 2×10^5 cells per well and incubated overnight. The following day, cells were pretreated with the indicated doses of ciglitazone (final concentrations) or vehicle control (DMSO) for 1 h before stimulation with heat-inactivated *S. aureus* (10^7 CFU/well) or PGN (10 μg/ml). At 24 h following bacterial stimulation, cell-free supernatants were collected and analyzed for IL-1β (*A*) and CXCL2 (*B*) expression. Microglial viability was assessed using a standard MTT assay, and the raw OD_{570} readings are reported (*C*). Results are reported as mean ± SD of three independent wells for each experimental treatment. **, $p < 0.001$ significant differences between microglia exposed to *S. aureus* or PGN compared with unstimulated cells. #, $p < 0.01$; ##, $p < 0.001$ significant differences between microglia exposed to *S. aureus* or PGN only vs cells pretreated with the various concentrations of ciglitazone plus *S. aureus* or PGN. Results are representative of four independent experiments.

Ciglitazone attenuates microglial proinflammatory mediator production in response to S. aureus *and its cell wall product PGN*

Microglia are the resident innate immune cells in the CNS parenchyma and are uniquely poised to immediately respond to bacterial infections by producing numerous cytokines and chemokines (42–44). Previous studies by our laboratory and others have revealed that PPAR-γ agonists are potent inhibitors of glial activation (18, 19, 45–48). However, our prior studies with microglia only examined responses to the endogenous PPAR-γ ligand 15d-PGJ_2 (18). Therefore, the ability of synthetic PPAR-γ agonists, such as ciglitazone, to modulate microglial responses to *S. aureus* has not yet been evaluated. Ciglitazone significantly reduced microglial IL-1β and CXCL2 production in response to *S. aureus* when administered before bacterial exposure, although considerable levels of each mediator remained (Fig. 9, *A* and *B*). Importantly, ciglitazone was still capable of attenuating *S. aureus*- and PGN-dependent microglial activation even when administered as late as 6 h following bacterial challenge (Fig. 10, *A* and *B*). The ability of ciglitazone to inhibit ongoing inflammation

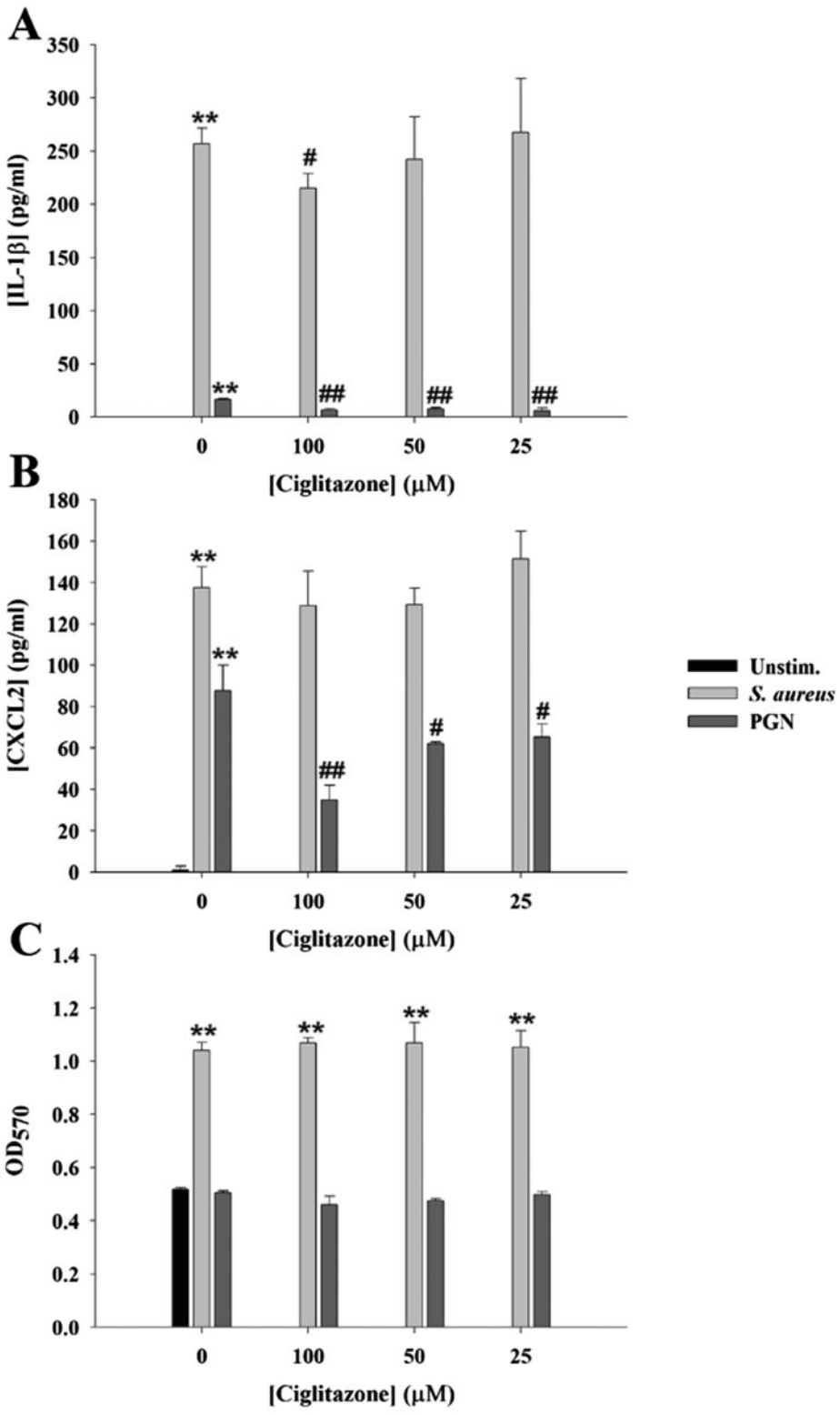

FIGURE 10. Ciglitazone is capable of attenuating ongoing proinflammatory mediator expression in microglia. Primary microglia were seeded in 96-well plates at 2×10^5 cells per well and incubated overnight. The following day, cells were left untreated or stimulated with heat-inactivated *S. aureus* (10^7 CFU/well) or PGN (10 μg/ml) for 6 h. Subsequently, microglia were treated with the indicated doses of ciglitazone (final concentrations) or vehicle control (DMSO). At 24 h following bacterial stimulation, cell-free supernatants were collected and analyzed for IL-1β (*A*) and CXCL2 (*B*) expression. Microglial viability was assessed using a standard MTT assay and the raw OD_{570} absorbance values are reported (*C*). Results are reported as mean ± SD of three independent wells for each experimental treatment. **, $p < 0.001$ significant differences between microglia exposed to *S. aureus* or PGN compared with unstimulated cells. #, $p < 0.01$; ##, $p < 0.001$ significant differences between microglia exposed to *S. aureus* or PGN only vs cells treated with the various concentrations of ciglitazone plus *S. aureus* or PGN. Results are representative of three independent experiments.

is important from a clinical standpoint because patients receiving treatment would already be experiencing overt CNS inflammatory responses. The reduction in proinflammatory mediator expression following ciglitazone treatment was not the result of overt cell death because MTT viability assays revealed that ciglitazone was not toxic to primary microglia at any of the doses examined (Figs. 9*C* and 10*C*). In fact, *S. aureus* exposure led to a significant increase in MTT readings regardless of ciglitazone treatment, suggestive of microglial proliferation. Collectively, these findings indicate that ciglitazone is able to modulate preexisting microglial activation, which to our knowledge, has not yet been reported in the literature.

Recent studies from our laboratory have demonstrated that brain abscesses are typified by a predominant Th1 response in an immunocompetent host (T. Kielian, J. Nichols, and N. Esen, manuscript in preparation). To determine whether ciglitazone may counteract the

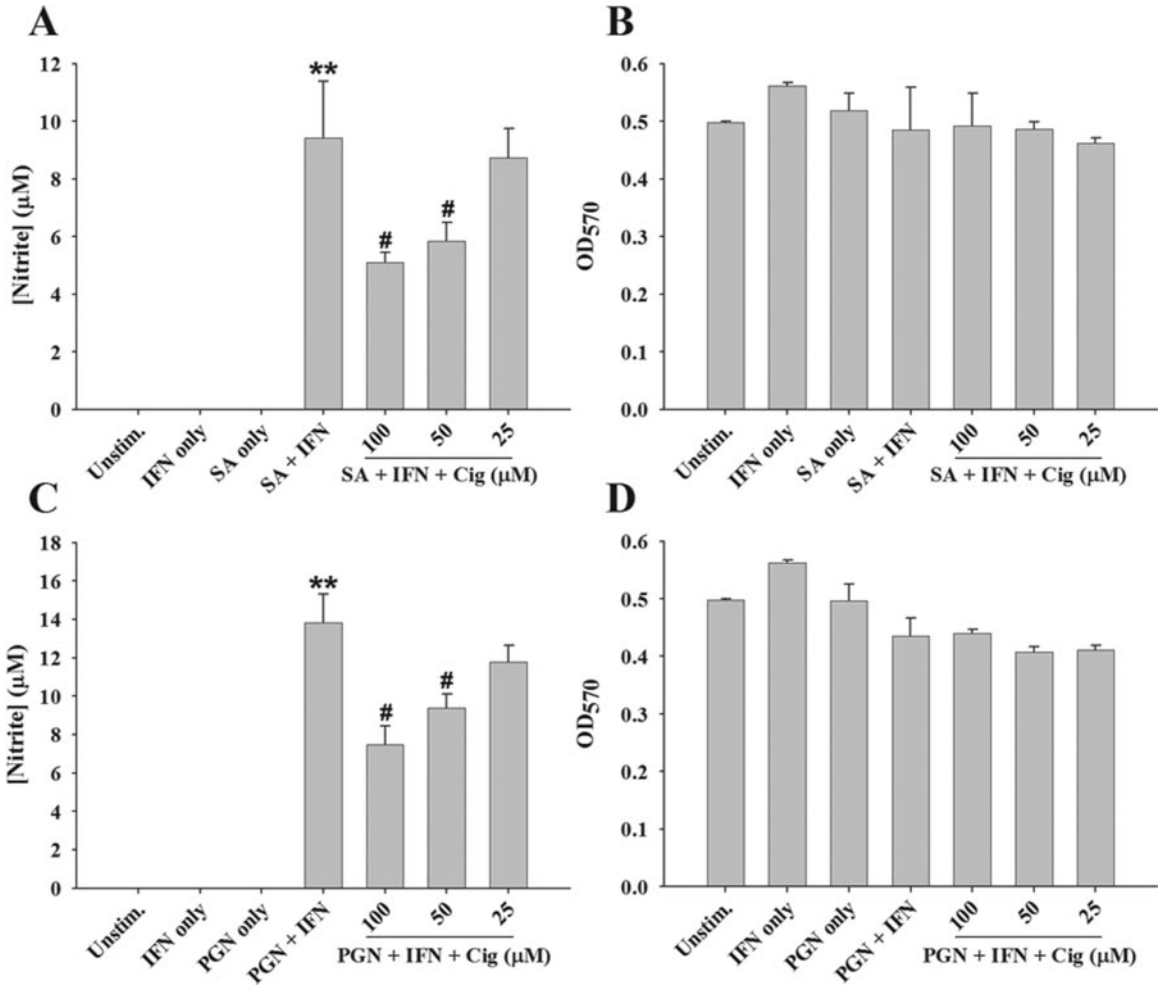

FIGURE 11. Ciglitazone interferes with the priming effects of IFN-γ in *S. aureus*-stimulated microglia. Primary microglia were seeded in 96-well plates at 2×10^5 cells per well and incubated overnight. The following day, cells were first pretreated with the indicated doses of ciglitazone (final concentrations) for 1 h followed by 100 ng/ml recombinant mouse IFN-γ for 1 h. Subsequently, microglia were stimulated with heat-inactivated *S. aureus* (10^7 CFU/well) (*A* and *B*) or PGN (10 μg/ml) (*C* and *D*). At 24 h following bacterial exposure, cell-free supernatants were collected and analyzed for NO expression (*A* and *C*). Microglial viability was assessed using a standard MTT assay and the raw OD_{570} readings are reported (*B* and *D*). Results are reported as mean ± SD of three independent wells for each experimental treatment. **, $p < 0.001$ significant differences between microglia exposed to *S. aureus* or PGN only compared with cells treated with bacterial stimuli plus IFN-γ. #, $p < 0.05$ significant differences between microglia exposed to *S. aureus* or PGN plus IFN-γ vs cells pretreated with the various concentrations of ciglitazone plus *S. aureus* or PGN plus IFN-γ. Results are representative of three independent experiments.

downstream effects of Th1 cytokines, we examined its ability to interfere with the immune potentiating effects of IFN-γ, a major product of Th1 cells. IFN-γ priming of primary microglia induced NO production in response to *S. aureus* or PGN stimulation, whereas neither bacterial stimulus alone was capable of eliciting detectable NO release (Fig. 11, *A* and *C*). Ciglitazone led to a significant reduction in NO production in response to IFN-γ plus *S. aureus* or PGN, suggesting that this PPAR-γ agonist may impact the nature of Th1-dependent responses during brain abscess development (Fig. 11, *A* and *C*). Cell viability assays revealed that the reductions in NO expression resulting from ciglitazone treatment were not due to cytotoxic effects (Fig. 11, *B* and *D*).

S. aureus *phagocytosis by microglia is enhanced by ciglitazone*

Phagocytosis of several particulate Ags, such as RBC and apoptotic cells, is enhanced by PPAR-γ agonists in microglia and macrophages (38, 39, 49). However, the ability of PPAR-γ agonists to modulate bacterial phagocytosis by microglia has not yet been investigated. Treatment of primary microglia with ciglitazone for a 24-h period before *S. aureus* exposure resulted in enhanced bacterial

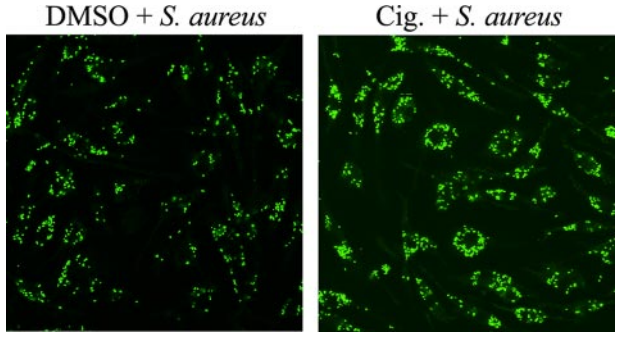

FIGURE 12. Ciglitazone enhances microglial phagocytosis of *S. aureus*. Primary microglia were seeded onto 12-mm coverslips at 2×10^5 cells per coverslip and incubated overnight in 24-well plates. The following day, cells were pretreated with 50 μM ciglitazone or vehicle (DMSO) for 24 h to influence PPAR-γ response element-driven gene expression. Subsequently, microglia were incubated with 4×10^6 heat-inactivated *S. aureus*-GFP (green) for 2 h, and visualization of intracellular bacteria was detected using confocal microscopy at a magnification ×40. Results are representative of three independent experiments.

uptake (Fig. 12). The ability of ciglitazone to augment *S. aureus* phagocytosis may explain, in part, why fewer viable bacteria were associated with brain abscesses of ciglitazone-treated mice (Fig. 3).

Discussion

There is no question that the host immune response elicited in the CNS parenchyma during brain abscess development is essential for effective bacterial containment. However, this response also contributes to the significant loss of brain tissue by necrosis. In addition, we have evidence to suggest that the immune response to bacteria during brain abscess evolution persists beyond the effective elimination of viable organisms from the parenchyma (22). This prolonged immune activation may be a consequence of continual triggering of pattern recognition receptors, such as TLRs (50, 51), by bacterial debris associated with the necrotic milieu including cell wall fragments and bacterial DNA. In essence, the CNS immunologically senses an infection without the presence of viable organisms. Under these conditions, we propose that the host immune response is deleterious, possibly confounding disease through the exaggerated destruction of noninfected brain parenchyma surrounding the abscess. Therefore, modulating the host CNS immune response to achieve effective bacterial neutralization while minimizing damage to surrounding tissue may be a useful strategy to improve the long-term prognosis for brain abscess survivors. Along these lines, our laboratory has recently demonstrated that minocycline, an antibiotic with anti-inflammatory properties, was capable of attenuating inflammation during brain abscess development independently of its bactericidal properties, culminating in a reduction in brain abscess size (26). To continue along this line of work, we next examined the ability of the PPAR-γ agonist ciglitazone to normalize the ensuing host inflammatory response for optimal bacterial killing with minimal bystander destruction to brain tissue.

The main finding of our study was that ciglitazone dramatically accelerated the development of the fibrotic brain abscess wall. This finding was revealed by enhanced fibronectin deposition and the earlier appearance of myofibroblasts expressing α-smooth muscle actin. This finding is at direct odds with other literature demonstrating that PPAR-γ agonists attenuate myofibroblast differentiation or fibrosis in models of pathological fibrosis such as scleroderma or pulmonary/hepatic fibrosis (52–56). However, it is important to acknowledge a fundamental difference between these models of excessive fibrosis vs brain abscess in which a highly regulated fibrotic wall forms in a region-specific manner. This fact, coupled with the well-known differences in tissue microenvironment between the brain and peripheral tissues (57, 58), may explain the pro-fibrotic effects of ciglitazone in the CNS compartment. However, because brain abscess is one of the few CNS insults that resolve by fibrosis rather than gliosis, it is likely that the pro-fibrotic effects of ciglitazone demonstrated in this study are specific to infection. This tenant is supported by a lack of evidence for pro-fibrotic responses in other CNS disorders in which PPAR-γ treatment has been used (15, 23–25). Another distinction between the regulated fibrosis that occurs during brain abscess development and pathological fibrosis is that the latter is typically associated with a Th2 cytokine environment (59, 60), which is not the case during brain abscess development where a Th1 response predominates (T. Kielian, unpublished observations). Indeed, IFN-γ, a major cytokine product of Th1 cells, is capable of potentiating NO release by *S. aureus*- or PGN-stimulated microglia and ciglitazone was found to interfere with the priming effects of IFN-γ. This difference in cytokine milieu also supports the argument that the reported effects of PPAR-γ agonists on inhibiting fibrosis in pathological models of disease are not reminiscent of what occurs during the controlled fibrosis associated with brain abscess wall formation. In addition, in terms of disease chronicity, brain abscesses typically last for weeks before treatment and resolution, which is quite distinct from the chronic disorders that PPAR-γ agonists have been reported to inhibit fibrosis (i.e., scleroderma, idiopathic pulmonary fibrosis, hepatic cirrhosis, and others) (55), which present themselves over months or years. Finally, another important concept to consider is that fibrotic responses typically ensue when inflammation is reduced (55). This relationship is in agreement with our findings, in that through its ability to attenuate proinflammatory mediator expression and bacterial burdens, ciglitazone enhances brain abscess encapsulation and fibrosis. Therefore, an alternative explanation to account for our results is that ciglitazone may not play an active role in fibrosis per se but rather, an indirect effect stemming from an earlier resolution of the host inflammatory response.

In models of wound healing in the skin, PPAR-γ is located in regions of active proliferation (i.e., along wound edges after injury) (61). Interestingly, the brain abscess wall is also located at the periphery of the lesion and demonstrates a great deal of vascular proliferative activity (7), theoretically making it a target region for ciglitazone action. Indeed, PPAR-γ-positive cells were clustered along the outer margins of the abscess and this finding provides strong correlative evidence that the effects of PPAR-γ on the developing abscess wall are mediated by the drug's action on this cell type. The identity of these PPAR-γ-positive cells remains to be demonstrated; however, based on cellular morphology and the characteristic pattern of microglial/macrophage aggregation along the abscess wall (7, 22), it is highly likely that these PPAR-γ-positive cells represent activated microglia/macrophages. Additional evidence demonstrating the impact of ciglitazone on this population was the ability of the drug to attenuate microglial/macrophage activation in brain abscesses as well as partially inhibit proinflammatory mediator expression in vitro. However, ciglitazone did not uniformly exert inhibitory actions on microglia as drug treatment enhanced *S. aureus* phagocytosis, which correlated with a reduction in bacterial burdens associated with brain abscesses. Therefore, our results indicate that ciglitazone has several immune modulatory effects on microglia/macrophages and may redirect their activation profile from proinflammatory to profibrotic. Indeed, both microglia and macrophages are capable of producing several fibrosis-promoting factors including TGF-β1 (62, 63). Although we did not detect any differences in PPAR-γ expression along the brain abscess wall in ciglitazone- vs vehicle-treated mice, there is one important distinction that requires clarification in comparison to other reports. Namely, we did not examine PPAR-γ expression in animals receiving a sterile sham injection vs those harboring brain abscesses because the objective of the current study was to localize PPAR-γ expression in the inflamed brain. It is likely that PPAR-γ levels are considerably increased following bacterial infection compared with the normal CNS as has been observed in other models of CNS inflammation (23). Therefore, our results simply provide an explanation for the localized actions of ciglitazone on the evolving brain abscess wall because PPAR-γ expression clusters in this region.

It is important to note that many anti-inflammatory actions of PPAR-γ agonists have been reported to occur via a receptor-independent manner through mechanisms such as transrepression of target genes and interference with signal transduction cascades (12, 19, 64–66). In contrast, in models of fibrosis, the actions of PPAR-γ ligands have, in large part, been shown to be receptor-dependent (52–54). Therefore, it is highly likely that ciglitazone may exert both receptor-dependent and -independent effects in

brain abscesses through its ability to accelerate fibrosis and inhibit inflammatory responses, respectively. It is not possible to investigate the role of PPAR-γ directly during brain abscess development because homozygous deletion of the PPAR-γ gene results in embryonic lethality (67, 68).

Flaris and Hickey (7) reported in a rat model of experimental brain abscess that the abscess wall was composed primarily of myofibroblasts based on their expression of type II collagen, fibronectin, and α-smooth muscle actin, in part similar to our findings in this study. Myofibroblasts can arise from the transdifferentiation of many different cell types including fibroblasts, smooth muscle cells, endothelial cells, and fibrocytes (8, 9). In peripheral organs such as the liver, kidney, and lung, myofibroblasts are thought to originate, in large part, from the transdifferentiation of resident fibroblasts under the control of cytokines, in particular TGF-β (8, 9, 60). However, the derivation of myofibroblasts from a fibroblast precursor during brain abscess development remains an enigma because the brain parenchyma is relatively devoid of fibroblasts, with the exception of those associated with the cerebral vasculature and the meninges. It is unlikely that meningeal fibroblasts contribute to the fibrotic reaction associated with brain abscesses because lesions are not contiguous with the meninges. In addition, a sterile stab wound injury to the brain parenchyma, which also perforates the meninges, does not lead to fibrosis (7, 22). Alternative possibilities to account for the origin of myofibroblasts along the developing brain abscess wall include transdifferentiation from smooth muscle cells, endothelial cells, or fibrocytes. The latter is a distinct population of blood-derived fibroblast-like progenitor cells that possess plasticity to differentiate along mesenchymal lineages, including commitment to myofibroblasts, adipocytes, and osteoblasts (69–72). Fibrocyte to myofibroblast transdifferentiation is an attractive possibility to account for the accumulation of myofibroblasts along the developing brain abscess wall because the extravasation of peripheral blood cells is a hallmark feature of brain abscess development, which continues out to at least 4 wk following infection (7, 22). The origin of myofibroblasts in the brain abscess model is currently being investigated in our laboratory.

Our findings describing the effects of ciglitazone on proinflammatory mediator expression in the brain abscess model differ slightly from previous studies investigating the role of PPAR-γ agonists in experimental autoimmune encephalomyelitis (EAE). Namely, we did not observe any alterations in IL-12 expression in brain abscesses with ciglitazone treatment, whereas others have reported that this compound is capable of reducing IL-12 levels during EAE (73). However, it is important to acknowledge several differences in the nature of inflammatory insults between these infectious (brain abscess) and noninfectious (i.e., EAE) disease models. For example, brain abscesses are typified by a robust neutrophil infiltrate (7, 21, 22), whereas these cells are not normally considered a hallmark of EAE. In addition, the voracity of the inflammatory response differs because a rapid, vigorous innate immune response is needed to contain bacterial replication during brain abscess development (3, 21, 30), whereas the intensity of the inflammatory response in EAE is limited to discrete foci. Based on the fundamental differences between these models of neuroinflammation, direct comparisons between the two paradigms are difficult at best. In addition to differences in model systems, other variables including the dose of PPAR-γ agonist administered and the interval when responses are measured (i.e., acute vs chronic inflammation) may influence the results obtained. It is important to note that in previous studies from our laboratory using purified microglia, we demonstrated that PPAR-γ agonists attenuated IL-12 p40 production in response to *S. aureus* stimulation (18). In addition, we noted that PPAR-γ agonists either had no effect or exacerbated CXCL2 production in primary microglia and astrocytes following *S. aureus* treatment, respectively (18, 19), the latter finding in agreement with other reports (74, 75). In summary, we propose that although PPAR-γ agonists have demonstrable effects on purified cell populations, these differences do not necessarily translate to the in vivo situation where a complex array of cell populations are encountered. Indeed, both CNS resident and several infiltrating immune cell types are associated with brain abscesses including microglia, astrocytes, macrophages, neutrophils, and T cells (6, 7, 22, 30, 76). Therefore, it appears reasonable that the effects observed with PPAR-γ agonists using purified cell populations may not always mimic the complex inflammatory milieu encountered during neuroinflammatory conditions such as brain abscess.

Another intriguing finding of this study was the ability of ciglitazone to augment *S. aureus* phagocytosis by microglia. We initially suspected that this effect may be mediated, in part, by the ability of PPAR-γ ligands to increase expression of the phagocytic class B scavenger receptor CD36 (38, 39). With regard to bacterial infection, CD36 has been shown to mediate the phagocytic uptake of *S. aureus* by macrophages (77). However, analysis of CD36 expression by quantitative RT-PCR did not reveal any significant alterations in receptor levels following ciglitazone treatment, indicating that the ability of this PPAR-γ agonist to augment *S. aureus* phagocytosis by microglia in unlikely to be explained by increased CD36 expression. Identifying the molecule(s) responsible for enhanced bacterial uptake is beyond the scope of this initial study characterizing the effects of ciglitazone on brain abscess development. Indeed, phagocytosis is a complex process involving many parallel signaling pathways and it is likely that multiple aspects of this process may be affected by ciglitazone. Future studies using microarray analysis could facilitate the identification of a candidate gene(s) involved in the phagocytic pathway that may be responsible for augmenting bacterial uptake in response to ciglitazone treatment.

In summary, our results provide evidence to suggest that the PPAR-γ agonist ciglitazone is capable of accelerating brain abscess encapsulation typified by the earlier appearance and compact organization of fibronectin as well as the rapid emergence of myofibroblasts associated with the developing abscess wall. This result could represent a protective drug response to control bacterial dissemination in the brain, an effect that is magnified by the ability of ciglitazone to significantly reduce abscess-associated bacterial burdens. Therefore, it is envisioned that combinational therapy with antibiotics and PPAR-γ agonists may achieve maximal benefit for the treatment of brain abscess patients by effectively reducing bacterial burdens and accelerating the protective encapsulation of abscesses.

Acknowledgments

We thank Rafi Kazi and Kelly McCastlain for excellent technical assistance.

Disclosures

The authors have no financial conflict of interest.

References

1. Mathisen, G. E., and J. P. Johnson. 1997. Brain abscess. *Clin. Infect. Dis.* 25: 763–781.
2. Townsend, G. C., and W. M. Scheld. 1998. Infections of the central nervous system. *Adv. Intern. Med.* 43: 403–447.
3. Kielian, T., E. D. Bearden, A. C. Baldwin, and N. Esen. 2004. IL-1 and TNF-α play a pivotal role in the host immune response in a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. *J. Neuropathol. Exp. Neurol.* 63: 381–396.

4. Kielian, T., A. Haney, P. M. Mayes, S. Garg, and N. Esen. 2005. Toll-like receptor 2 modulates the proinflammatory milieu in *Staphylococcus aureus*-induced brain abscess. *Infect. Immun.* 73: 7428–7435.
5. Kielian, T., N. K. Phulwani, N. Esen, M. M. Syed, A. C. Haney, K. McCastlain, and J. Johnson. 2007. MyD88-dependent signals are essential for the host immune response in experimental brain abscess. *J. Immunol.* 178: 4528–4537.
6. Kielian, T. 2004. Immunopathogenesis of brain abscess. *J. Neuroinflammation.* 1: 16.
7. Flaris, N. A., and W. F. Hickey. 1992. Development and characterization of an experimental model of brain abscess in the rat. *Am. J. Pathol.* 141: 1299–1307.
8. Hinz, B., S. H. Phan, V. J. Thannickal, A. Galli, M. L. Bochaton-Piallat, and G. Gabbiani. 2007. The myofibroblast: one function, multiple origins. *Am. J. Pathol.* 170: 1807–1816.
9. Hinz, B. 2007. Formation and function of the myofibroblast during tissue repair. *J. Invest. Dermatol.* 127: 526–537.
10. Davis, L. E., and N. G. Baldwin. 1999. Brain abscess. *Curr. Treat. Options Neurol.* 1: 157–166.
11. Lu, C. H., W. N. Chang, and C. C. Lui. 2006. Strategies for the management of bacterial brain abscess. *J. Clin. Neurosci.* 13: 979–985.
12. Kielian, T., and P. D. Drew. 2003. Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation. *J. Neurosci. Res.* 71: 315–325.
13. Feige, J. N., L. Gelman, L. Michalik, B. Desvergne, and W. Wahli. 2006. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. *Prog. Lipid Res.* 45: 120–159.
14. Kostadinova, R., W. Wahli, and L. Michalik. 2005. PPARs in diseases: control mechanisms of inflammation. *Curr. Med. Chem.* 12: 2995–3009.
15. Heneka, M. T., and G. E. Landreth. 2007. PPARs in the brain. *Biochim. Biophys. Acta* 1771: 1031–1045.
16. Drew, P. D., J. Xu, P. D. Storer, J. A. Chavis, and M. K. Racke. 2006. Peroxisome proliferator-activated receptor agonist regulation of glial activation: relevance to CNS inflammatory disorders. *Neurochem. Int.* 49: 183–189.
17. Bernardo, A., and L. Minghetti. 2006. PPAR-γ agonists as regulators of microglial activation and brain inflammation. *Curr. Pharm. Des.* 12: 93–109.
18. Kielian, T., M. McMahon, E. D. Bearden, A. C. Baldwin, P. D. Drew, and N. Esen. 2004. *S. aureus*-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2). *J. Neurochem.* 90: 1163–1172.
19. Phulwani, N. K., D. L. Feinstein, V. Gavrilyuk, C. Akar, and T. Kielian. 2006. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and ciglitazone modulate *Staphylococcus aureus*-dependent astrocyte activation primarily through a PPAR-γ-independent pathway. *J. Neurochem.* 99: 1389–1402.
20. Kielian, T., A. Cheung, and W. F. Hickey. 2001. Diminished virulence of an α-toxin mutant of *Staphylococcus aureus* in experimental brain abscesses. *Infect. Immun.* 69: 6902–6911.
21. Kielian, T., B. Barry, and W. F. Hickey. 2001. CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. *J. Immunol.* 166: 4634–4643.
22. Baldwin, A. C., and T. Kielian. 2004. Persistent immune activation associated with a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. *J. Neuroimmunol.* 151: 24–32.
23. Diab, A., C. Deng, J. D. Smith, R. Z. Hussain, B. Phanavanh, A. E. Lovett-Racke, P. D. Drew, and M. K. Racke. 2002. Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis. *J. Immunol.* 168: 2508–2515.
24. Feinstein, D. L., E. Galea, V. Gavrilyuk, C. F. Brosnan, C. C. Whitacre, L. Dumitrescu-Ozimek, G. E. Landreth, H. A. Pershadsingh, G. Weinberg, and M. T. Heneka. 2002. Peroxisome proliferator-activated receptor-γ agonists prevent experimental autoimmune encephalomyelitis. *Ann. Neurol.* 51: 694–702.
25. Heneka, M. T., M. Sastre, L. Dumitrescu-Ozimek, A. Hanke, I. Dewachter, C. Kuiperi, K. O'Banion, T. Klockgether, F. Van Leuven, and G. E. Landreth. 2005. Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1–42 levels in APPV717I transgenic mice. *Brain* 128(Pt. 6): 1442–1453.
26. Kielian, T., N. Esen, S. Liu, N. K. Phulwani, M. M. Syed, N. Phillips, K. Nishina, A. L. Cheung, J. D. Schwartzman, and J. J. Ruhe. 2007. Minocycline modulates neuroinflammation independently of its antimicrobial activity in *Staphylococcus aureus*-induced brain abscess. *Am. J. Pathol.* 171: 1199–1214.
27. Esen, N., F. Y. Tanga, J. A. DeLeo, and T. Kielian. 2004. Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the Gram-positive bacterium *Staphylococcus aureus*. *J. Neurochem.* 88: 746–758.
28. Esen, N., and T. Kielian. 2006. Central role for MyD88 in the responses of microglia to pathogen-associated molecular patterns. *J. Immunol.* 176: 6802–6811.
29. Esen, N., and T. Kielian. 2007. Effects of low dose GM-CSF on microglial inflammatory profiles to diverse pathogen-associated molecular patterns (PAMPs). *J. Neuroinflammation* 4: 10.
30. Stenzel, W., S. Soltek, H. Miletic, M. M. Hermann, H. Korner, J. D. Sedgwick, D. Schluter, and M. Deckert. 2005. An essential role for tumor necrosis factor in the formation of experimental murine *Staphylococcus aureus*-induced brain abscess and clearance. *J. Neuropathol. Exp. Neurol.* 64: 27–36.
31. McCoy, M. K., T. N. Martinez, K. A. Ruhn, D. E. Szymkowski, C. G. Smith, B. R. Botterman, K. E. Tansey, and M. G. Tansey. 2006. Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. *J. Neurosci.* 26: 9365–9375.
32. Li, R., L. Yang, K. Lindholm, Y. Konishi, X. Yue, H. Hampel, D. Zhang, and Y. Shen. 2004. Tumor necrosis factor death receptor signaling cascade is required for amyloid-β protein-induced neuron death. *J. Neurosci.* 24: 1760–1771.
33. Hovelmeyer, N., Z. Hao, K. Kranidioti, G. Kassiotis, T. Buch, F. Frommer, L. von Hoch, D. Kramer, L. Minichiello, G. Kollias, et al. 2005. Apoptosis of oligodendrocytes via Fas and TNF-R1 is a key event in the induction of experimental autoimmune encephalomyelitis. *J. Immunol.* 175: 5875–5884.
34. Lehnardt, S., C. Lachance, S. Patrizi, S. Lefebvre, P. L. Follett, F. E. Jensen, P. A. Rosenberg, J. J. Volpe, and T. Vartanian. 2002. The Toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J. Neurosci.* 22: 2478–2486.
35. Jacobs, C. A., P. E. Baker, E. R. Roux, K. S. Picha, B. Toivola, S. Waugh, and M. K. Kennedy. 1991. Experimental autoimmune encephalomyelitis is exacerbated by IL-1α and suppressed by soluble IL-1 receptor. *J. Immunol.* 146: 2983–2989.
36. Sutton, C., C. Brereton, B. Keogh, K. H. Mills, and E. C. Lavelle. 2006. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J. Exp. Med.* 203: 1685–1691.
37. MacMicking, J., Q. W. Xie, and C. Nathan. 1997. Nitric oxide and macrophage function. *Annu. Rev. Immunol.* 15: 323–350.
38. Zhao, X., G. Sun, J. Zhang, R. Strong, W. Song, N. Gonzales, J. C. Grotta, and J. Aronowski. 2007. Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor γ in microglia/macrophages. *Ann. Neurol.* 61: 352–362.
39. Moore, K. J., E. D. Rosen, M. L. Fitzgerald, F. Randow, L. P. Andersson, D. Altshuler, D. S. Milstone, R. M. Mortensen, B. M. Spiegelman, and M. W. Freeman. 2001. The role of PPAR-γ in macrophage differentiation and cholesterol uptake. *Nat. Med.* 7: 41–47.
40. Darby, I., O. Skalli, and G. Gabbiani. 1990. α-Smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab. Invest.* 63: 21–29.
41. Masur, S. K., H. S. Dewal, T. T. Dinh, I. Erenburg, and S. Petridou. 1996. Myofibroblasts differentiate from fibroblasts when plated at low density. *Proc. Natl. Acad. Sci. USA* 93: 4219–4223.
42. Aloisi, F. 2001. Immune function of microglia. *Glia* 36: 165–179.
43. Hanisch, U. K. 2002. Microglia as a source and target of cytokines. *Glia* 40: 140–155.
44. Kielian, T. 2004. Microglia and chemokines in infectious diseases of the nervous system: views and reviews. *Front. Biosci.* 9: 732–750.
45. Drew, P. D., and J. A. Chavis. 2001. The cyclopentone prostaglandin 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 represses nitric oxide, TNF-α, and IL-12 production by microglial cells. *J. Neuroimmunol.* 115: 28–35.
46. Xu, J., and P. D. Drew. 2007. Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia. *J. Immunol.* 178: 1904–1913.
47. Luna-Medina, R., M. Cortes-Canteli, M. Alonso, A. Santos, A. Martínez, and A. Perez-Castillo. 2005. Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor γ activation. *J. Biol. Chem.* 280: 21453–21462.
48. Storer, P. D., J. Xu, J. Chavis, and P. D. Drew. 2005. Peroxisome proliferator-activated receptor-γ agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis. *J. Neuroimmunol.* 161: 113–122.
49. Majai, G., Z. Sarang, K. Csomos, G. Zahuczky, and L. Fesus. 2007. PPARγ-dependent regulation of human macrophages in phagocytosis of apoptotic cells. *Eur. J. Immunol.* 37: 1343–1354.
50. Akira, S. 2006. TLR signaling. *Curr. Top. Microbiol. Immunol.* 311: 1–16.
51. Kielian, T. 2006. Toll-like receptors in central nervous system glial inflammation and homeostasis. *J. Neurosci. Res.* 83: 711–730.
52. Galli, A., D. W. Crabb, E. Ceni, R. Salzano, T. Mello, G. Svegliati-Baroni, F. Ridolfi, L. Trozzi, C. Surrenti, and A. Casini. 2002. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. *Gastroenterology* 122: 1924–1940.
53. Hong, K. M., J. A. Belperio, M. P. Keane, M. D. Burdick, and R. M. Strieter. 2007. Differentiation of human circulating fibrocytes as mediated by transforming growth factor-β and peroxisome proliferator-activated receptor γ. *J. Biol. Chem.* 282: 22910–22920.
54. Ghosh, A. K., S. Bhattacharyya, G. Lakos, S. J. Chen, Y. Mori, and J. Varga. 2004. Disruption of transforming growth factor β signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor γ. *Arthritis Rheum.* 50: 1305–1318.
55. Michalik, L., and W. Wahli. 2006. Involvement of PPAR nuclear receptors in tissue injury and wound repair. *J. Clin. Invest.* 116: 598–606.
56. Burgess, H. A., L. E. Daugherty, T. H. Thatcher, H. F. Lakatos, D. M. Ray, M. Redonnet, R. P. Phipps, and P. J. Sime. 2005. PPARγ agonists inhibit TGF-β induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis. *Am. J. Physiol.* 288: L1146–L1153.
57. Carson, M. J., J. M. Doose, B. Melchior, C. D. Schmid, and C. C. Ploix. 2006. CNS immune privilege: hiding in plain sight. *Immunol. Rev.* 213: 48–65.
58. Wekerle, H. 2006. Breaking ignorance: the case of the brain. *Curr. Top. Microbiol. Immunol.* 305: 25–50.
59. Wynn, T. A. 2004. Fibrotic disease and the T_H1/T_H2 paradigm. *Nat. Rev. Immunol.* 4: 583–594.
60. Lupher, M. L., Jr., and W. M. Gallatin. 2006. Regulation of fibrosis by the immune system. *Adv. Immunol.* 89: 245–288.
61. Michalik, L., B. Desvergne, N. S. Tan, S. Basu-Modak, P. Escher, J. Rieusset, J. M. Peters, G. Kaya, F. J. Gonzalez, J. Zakany, et al. 2001. Impaired skin wound

healing in peroxisome proliferator-activated receptor (PPAR)α and PPARβ mutant mice. *J. Cell Biol.* 154: 799–814.
62. Li, M. O., Y. Y. Wan, S. Sanjabi, A. K. Robertson, and R. A. Flavell. 2006. Transforming growth factor-β regulation of immune responses. *Annu. Rev. Immunol.* 24: 99–146.
63. Bottner, M., K. Krieglstein, and K. Unsicker. 2000. The transforming growth factor-βs: structure, signaling, and roles in nervous system development and functions. *J. Neurochem.* 75: 2227–2240.
64. Pascual, G., and C. K. Glass. 2006. Nuclear receptors versus inflammation: mechanisms of transrepression. *Trends Endocrinol. Metab.* 17: 321–327.
65. Chawla, A., Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans. 2001. PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat. Med.* 7: 48–52.
66. Pascual, G., A. L. Fong, S. Ogawa, A. Gamliel, A. C. Li, V. Perissi, D. W. Rose, T. M. Willson, M. G. Rosenfeld, and C. K. Glass. 2005. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ. *Nature* 437: 759–763.
67. Barak, Y., M. C. Nelson, E. S. Ong, Y. Z. Jones, P. Ruiz-Lozano, K. R. Chien, A. Koder, and R. M. Evans. 1999. PPARγ is required for placental, cardiac, and adipose tissue development. *Mol. Cell* 4: 585–595.
68. Kubota, N., Y. Terauchi, H. Miki, H. Tamemoto, T. Yamauchi, K. Komeda, S. Satoh, R. Nakano, C. Ishii, T. Sugiyama, et al. 1999. PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* 4: 597–609.
69. Hong, K. M., M. D. Burdick, R. J. Phillips, D. Heber, and R. M. Strieter. 2005. Characterization of human fibrocytes as circulating adipocyte progenitors and the formation of human adipose tissue in SCID mice. *FASEB J.* 19: 2029–2031.
70. Phillips, R. J., M. D. Burdick, K. Hong, M. A. Lutz, L. A. Murray, Y. Y. Xue, J. A. Belperio, M. P. Keane, and R. M. Strieter. 2004. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J. Clin. Invest.* 114: 438–446.
71. Abe, R., S. C. Donnelly, T. Peng, R. Bucala, and C. N. Metz. 2001. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J. Immunol.* 166: 7556–7562.
72. Bucala, R., L. A. Spiegel, J. Chesney, M. Hogan, and A. Cerami. 1994. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol. Med.* 1: 71–81.
73. Natarajan, C., and J. J. Bright. 2002. Peroxisome proliferator-activated receptor-γ agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation. *Genes Immun.* 3: 59–70.
74. Zhang, X., J. M. Wang, W. H. Gong, N. Mukaida, and H. A. Young. 2001. Differential regulation of chemokine gene expression by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2. *J. Immunol.* 166: 7104–7111.
75. Si, Q., M. L. Zhao, A. C. Morgan, C. F. Brosnan, and S. C. Lee. 2004. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits IFN-inducible protein 10/CXC chemokine ligand 10 expression in human microglia: mechanisms and implications. *J. Immunol.* 173: 3504–3513.
76. Stenzel, W., S. Soltek, D. Schluter, and M. Deckert. 2004. The intermediate filament GFAP is important for the control of experimental murine *Staphylococcus aureus*-induced brain abscess and *Toxoplasma encephalitis*. *J. Neuropathol. Exp. Neurol.* 63: 631–640.
77. Stuart, L. M., J. Deng, J. M. Silver, K. Takahashi, A. A. Tseng, E. J. Hennessy, R. A. Ezekowitz, and K. J. Moore. 2005. Response to *Staphylococcus aureus* requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. *J. Cell Biol.* 170: 477–485.