

# *Escherichia coli* K1 Modulates Peroxisome Proliferator-Activated Receptor $\gamma$ and Glucose Transporter 1 at the Blood-Brain Barrier in Neonatal Meningitis

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*Escherichia coli* K1 meningitis continues to be a major threat to neonatal health. Previous studies demonstrated that outer membrane protein A (OmpA) of *E. coli* K1 interacts with endothelial cell glycoprotein 96 (Ecg96) in the blood-brain barrier to enter the central nervous system. Here we show that the interaction between OmpA and Ecg96 downregulates peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) and glucose transporter 1 (GLUT-1) levels in human brain microvascular endothelial cells, causing disruption of barrier integrity and inhibition of glucose uptake. The suppression of PPAR- $\gamma$  and GLUT-1 by the bacteria in the brain microvessels of newborn mice causes extensive pathophysiology owing to interleukin 6 production. Pretreatment with partial or selective PPAR- $\gamma$  agonists ameliorate the pathological outcomes of infection by suppressing interleukin 6 production in the brain. Thus, inhibition of PPAR- $\gamma$  and GLUT-1 by *E. coli* K1 is a novel pathogenic mechanism in meningitis, and pharmacological upregulation of PPAR- $\gamma$  and GLUT-1 levels may provide novel therapeutic avenues.

**Keywords.** *Escherichia coli* K1; meningitis, blood-brain barrier; PPAR- $\gamma$ ; GLUT-1; glucose uptake.

Bacterial meningitis is a serious condition that affects the central nervous system. Neonatal and childhood meningitis in particular result in long-term neurological sequelae in about 50% of the survivors [1]. Despite the advent of vaccines and effective antibiotic treatment, clonal variants and antibiotic resistance have recently emerged [2]. One of the central yet incompletely understood dogmas of bacterial meningitis is the reduced glucose levels in the cerebrospinal fluid (CSF) of patients. The CSF glucose levels are typically correlated to serum glucose levels since glucose can be transported across the blood-brain barrier (BBB). A recent clinical study with 3805 healthy volunteers with a median age of 2.2 months showed that increased serum glucose levels correlated with increased CSF glucose levels. Therefore, the BBB is the focal point for glucose transport from the blood to the CSF. During bacterial meningitis, the requirement of glucose as a fuel by infiltrating immune cells in response to infection is considered a reason for the reduced glucose levels [3, 4]. However, the possibility of whether bacterial pathogens that cause meningitis can manipulate glucose concentrations in the brain has not been explored.

*Escherichia coli* K1 is the second leading cause of neonatal meningitis, and it uses distinct cellular mechanisms to cross the BBB for disease progression [5]. There is a reemergence of this pathogen with atypical pathogenic mechanisms [6, 7]. Therefore, it is imperative that our understanding of this pathogen is abreast with its ever-changing virulence strategies. We unequivocally established that the outer membrane protein A (OmpA) of *E. coli* K1 binds endothelial cell glycoprotein 96 (Ecg96) in both human brain microvascular endothelial cells (HBMECs), an in vitro model of the BBB, and in a newborn mouse model of meningitis. This interaction is critical for *E. coli* K1 to cross the BBB and establish infection [8–10]. Ecg96 belongs to the glucose-regulated protein 94 family of heat shock proteins, and therefore its expression is typically induced by lack of glucose or glucose starvation [11]. Our studies also demonstrated the existence of a feedback loop between Ecg96/Toll-like receptor (TLR)/angiotensin II receptor I (AT1R) protein complex in the membranes of HBMECs and intracellular nitric oxide during the invasion process [12–14].

The brain is a glucose-dependent organ because fatty acids cannot cross the BBB [15]. The peroxisome proliferator-activating receptor (PPAR) superfamily of nuclear receptors plays a vital role in cellular glucose uptake by promoting translocation of glucose transporter 1 (GLUT-1) to the membrane and facilitating glucose uptake into the brain from blood vessels [16–18]. Modulation of PPAR- $\gamma$  and GLUT-1 expression is critical in neurodegenerative disorders [19–24]. Interestingly, the AT1R antagonist telmisartan (TS), which effectively blocked *E. coli* K1 invasion in vitro and in vivo, also acts as a partial agonist

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for PPAR- $\gamma$  activation [14, 25]. Therefore, we speculated that the initial OmpA binding to Ecgp96 triggers a glucose-deficient environment that induces more Ecgp96 expression for bacterial binding and invasion. In this study, we sought to examine the roles of PPAR- $\gamma$  and GLUT-1 in *E. coli* K1 invasion of HBMECs and the onset of meningitis in newborn mice.

## METHODS

### Strains and Reagents

The prototype *E. coli* K1 strain (O18:K1:H7) and its *ompA* deletion mutant were described previously [26]. The list of antibodies and chemicals used in this study are described in detail in the Supplementary Materials.

### Cell Culture and Infection Assays

HBMEC cultures and infection protocols were performed as described elsewhere [27]. HBMECs were pretreated with the respective compounds for 1 hour before infection. For protein overexpression, HBMECs were transfected with the respective plasmids, and invasion assays were performed 24 hours after transfection.

### Animal Studies, Immunostaining, and Cytokine Enzyme-Linked Immunosorbent Assay (ELISA)

Animal experiments were performed as described previously [26]. Protocols were approved by the Institutional Animal Care and Use Committee of Children's Hospital of Los Angeles (protocol 59–14). For treatment studies, the pups were treated with TS or rosiglitazone (RG) 6 hours before infection, during infection, and 18 hours after infection. Details are provided in the Supplementary Materials. Cytokine ELISA was performed as per the manufacturer's protocol. Hematoxylin and eosin (HE), immunofluorescence, and brain microvessel staining were executed as reported before [14, 24, 26].

### Other Methods

Western blotting using HBMEC lysates, flow cytometry, and measurement of trans-endothelial electrical resistance were performed as described earlier [14]. Glucose measurement assays were performed as described earlier [28].

## RESULTS

### *E. coli* K1 Suppresses PPAR- $\gamma$ and GLUT-1 During the Invasion of HBMECs

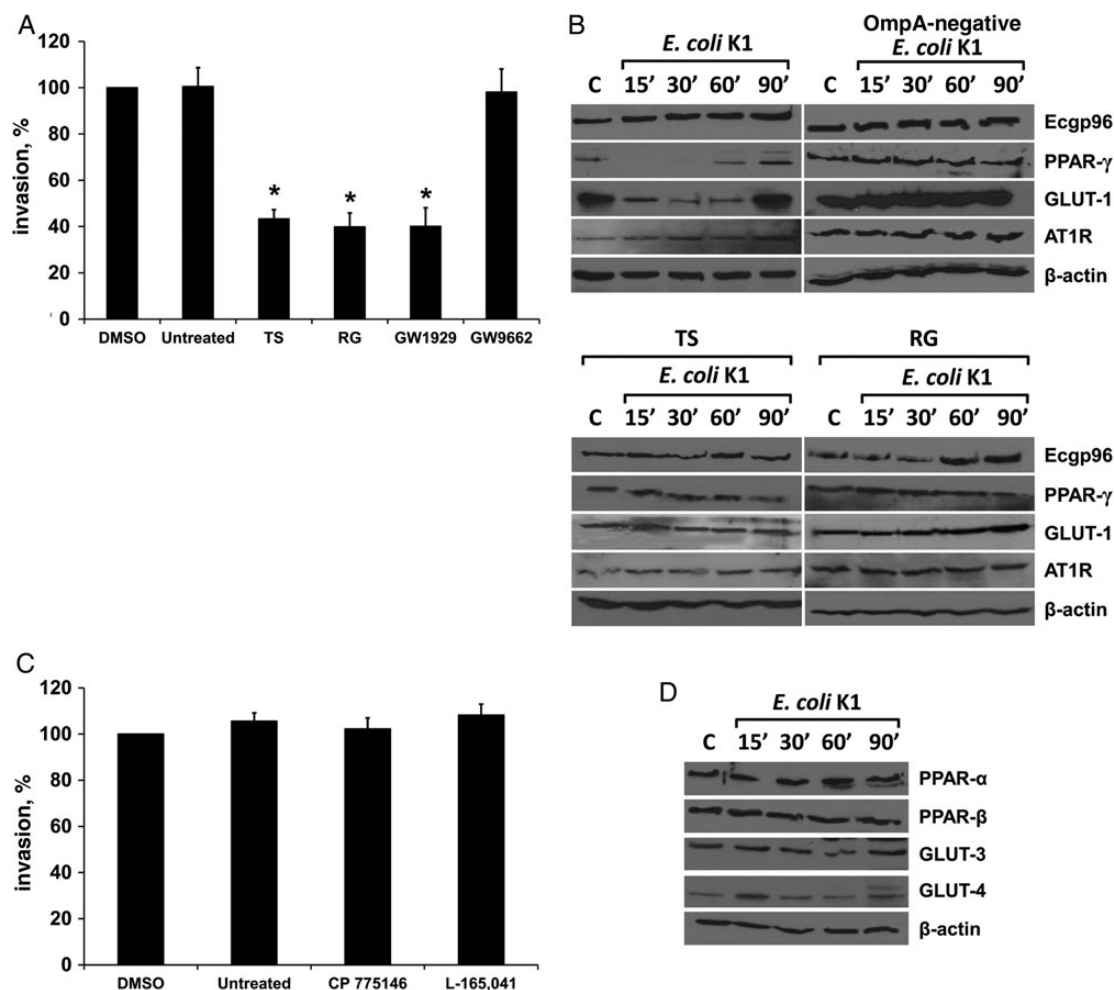
To examine whether PPAR- $\gamma$  and GLUT-1 play a role in *E. coli* K1 entry of HBMECs, invasion assays were performed in the presence of TS (partial PPAR- $\gamma$  agonist/antihypertensive drug), RG (selective PPAR- $\gamma$  agonist/anti-diabetic drug), GW1929 (selective PPAR- $\gamma$  agonist), and GW9662 (PPAR- $\gamma$  antagonist). The half maximal inhibitory concentrations were determined using dose-dependent studies prior to these experiments (data not shown). Results revealed that pretreatment of HBMECs with TS, RG, and GW1929 blocked *E. coli* K1 invasion, while pretreatment with PPAR- $\gamma$  antagonist GW9662 did

not have any effect on invasion (Figure 1A). Western blot analysis of *E. coli* K1-infected HBMEC lysates showed that PPAR- $\gamma$  expression was suppressed until 30 minutes and recovered by 90 minutes after infection (Figure 1B). The expression of GLUT-1 was also significantly suppressed in *E. coli* K1-infected cells, as shown by immunoblotting of the infected HBMEC lysates. As previously demonstrated, the expression of Ecgp96, the receptor for *E. coli* K1 OmpA, increased with infection. The expression of AT1R, previously established to be important in the invasion process, did not change with infection. This observation was also consistent with our previous findings that total AT1R levels in HBMECs do not change with *E. coli* K1 invasion but that the association of AT1R with Ecgp96 increases during the invasion process [14]. No such changes were observed in HBMECs infected with OmpA-negative *E. coli* K1.

TS or RG pretreatment did not significantly alter Ecgp96 or AT1R expression after *E. coli* K1 infection of HBMECs, compared with cells infected with *E. coli* K1 alone. In contrast, pretreatment with TS or RG inhibited *E. coli* K1-mediated suppression of PPAR- $\gamma$  and GLUT-1, suggesting that suppression of these 2 molecules plays a role in the invasion of *E. coli* K1 in HBMECs. To rule out the possible involvement of other PPAR isoforms, we performed invasion assays on HBMECs pretreated with PPAR- $\alpha$  agonist CP 775146 and PPAR- $\beta$  agonist L-165 041. As shown in Figure 1C, both agonists had no effect on the invasion process. Furthermore, to determine whether PPAR- $\alpha$  and PPAR- $\beta$  expression is affected in *E. coli* K1-infected HBMECs, Western blots were performed using total cell lysates. Both isoforms were expressed in detectable amounts in the lysates, but the infection process did not alter their levels (Figure 1D). Additionally, the lysates were also examined for the expression of GLUT-3 and GLUT-4 since both these transporters have been identified at basal levels in the BBB of both humans and rodents [15, 29]. Consistent with this observation, basal levels of GLUT-3 and GLUT-4 were detected in HBMEC lysates, but their levels remained fairly unchanged during the invasion. These results confirm that *E. coli* K1 invasion of HBMECs suppresses the expression of PPAR- $\gamma$  and GLUT-1. The effect of GLUT-1 agonists on *E. coli* K1 invasion could not be tested due to lack of commercially available compounds that exclusively upregulate GLUT-1. To circumvent this issue, we performed invasion studies in HBMECs overexpressing PPAR- $\gamma$  and GLUT-1.

### Overexpression of Recombinant PPAR- $\gamma$ or GLUT-1 Blocks *E. coli* K1 Invasion of HBMECs

To confirm the role of PPAR- $\gamma$  and GLUT-1 in *E. coli* K1 entry of HBMECs, plasmids overexpressing full-length PPAR- $\gamma$  (FL-PPAR- $\gamma$ ) or GLUT-1 (FL-GLUT-1) were transiently transfected in HBMECs, and invasion assays were performed as described above. HBMECs overexpressing full-length GLUT-4 (FL-GLUT-4) were used as a control. Since GLUT-4-mediated



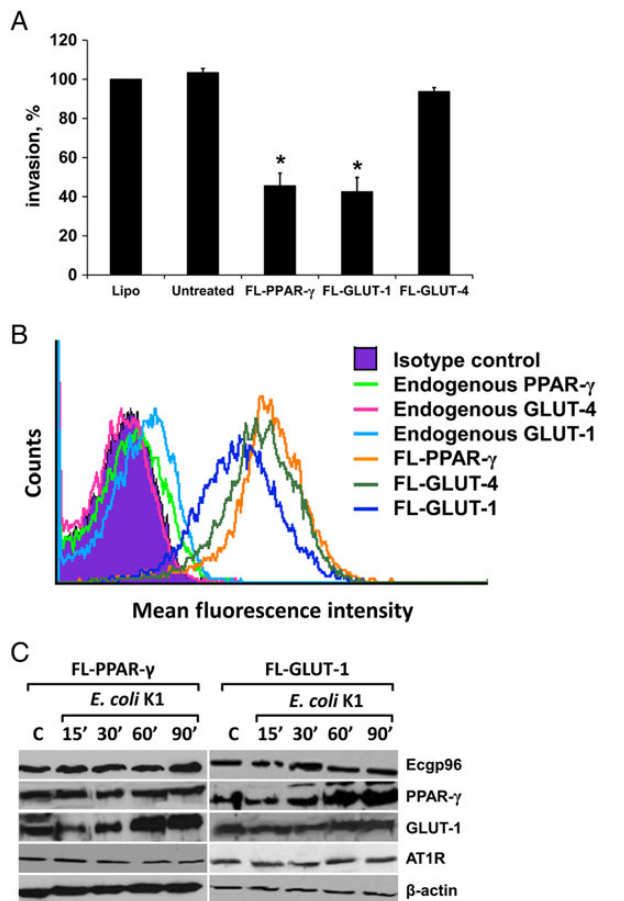
**Figure 1.** *Escherichia coli* K1 suppresses peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) and glucose transporter 1 (GLUT-1) expression during the invasion of human brain microvascular endothelial cells (HBMECs). **A**, Bacterial invasion assays in HBMECs were performed using the following concentrations of telmisartan (TS; 39  $\mu$ M), rosiglitazone (RG; 50  $\mu$ M), GW1929 (50  $\mu$ M), and GW9662 (33 nM). Data represent relative invasion as compared to *E. coli* K1 in the presence of dimethyl sulfoxide (DMSO), taken as 100%, and are averages of 3 independent experiments. Error bars indicate standard deviation. \* $P < .05$  by Student *t* test as compared to DMSO. **B**, Lysates from HBMECs exposed to bacteria for up to 90 minutes in the presence or absence of compound pretreatment for 1 hour were probed for expression of endothelial cell glycoprotein 96 (Ecgp96), PPAR- $\gamma$ , GLUT-1, and angiotensin II receptor I (AT1R).  $\beta$ -actin was used as a loading control; "C" indicates uninfected control. The blots shown are representative of 2 independent experiments with similar results. **C**, Bacterial invasion assays on HBMECs were performed using the PPAR- $\alpha$  agonist CP 775146 (24.5 nM) or the PPAR- $\beta$  agonist L-165 041 (125 nM). Data represent relative invasion as compared to *E. coli* K1 in the presence of DMSO, taken as 100%, and are averages of 3 independent experiments. Error bars indicate standard deviation. **D**, Expression levels of PPAR- $\alpha$ , PPAR- $\beta$ , GLUT-3, and GLUT-4 were analyzed from lysates of HBMECs infected with *E. coli* K1 for various time points as indicated.  $\beta$ -actin was used as a loading control; "C" indicates uninfected control. The blots shown are representative of 2 independent experiments that exhibited similar results. OmpA, outer membrane protein A.

glucose uptake is strictly insulin dependent, it was expected to play no role in glucose uptake in infected HBMECs [15]. *E. coli* K1 invasion was reduced by >50% in HBMECs overexpressing FL-PPAR- $\gamma$  or FL-GLUT-1, while its invasion of HBMECs overexpressing FL-GLUT-4 was comparable to invasion of untransfected or transfection reagent alone-treated HBMECs (Figure 2A). The overexpression of these proteins in transfected HBMECs were also comparable, as verified by flow cytometry (Figure 2B). Western blot analyses of lysates from transfected HBMECs infected with *E. coli* K1 showed that the expressions of Ecgp96, AT1R, PPAR- $\gamma$ , and GLUT-1 were similar to those

observed with RG-pretreated/*E. coli* K1-infected HBMECs (Figure 2C). These observations confirm that upregulation of PPAR- $\gamma$  and GLUT-1 levels either by RG pretreatment or with plasmid-mediated overexpression blocks *E. coli* K1 invasion.

#### ***E. coli* K1 Suppresses Glucose Uptake and Induces Tight Junction Disruption in HBMECs During the Invasion**

To demonstrate the direct role of glucose uptake in HBMECs in preventing *E. coli* K1 invasion, a colorimetric assay to quantify 2-deoxyglucose uptake was performed [28]. *E. coli* K1 suppressed glucose uptake in HBMECs in comparison to uninfected control



**Figure 2.** Overexpression of recombinant peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) or glucose transporter 1 (GLUT-1) blocks *Escherichia coli* K1 invasion of human brain microvascular endothelial cells (HBMECs). **A**, HBMECs transfected with plasmids expressing full-length (FL)-PPAR- $\gamma$ , FL-GLUT-1, or FL-GLUT-4 were subjected to invasion assays with *E. coli* K1. Data represent relative invasion as compared to *E. coli* K1 in the presence of Lipofectamine 3000 (Lipo), taken as 100%, and are averages of 3 independent experiments. Error bars indicate standard deviation. \* $P < .05$  by the Student  $t$  test as compared to Lipofectamine 3000 (Lipo). **B**, Flow cytometry of untransfected and plasmid-transfected HBMECs was performed using respective antibodies. Data are representative of 2 independent experiments with similar results. **C**, Lysates from untransfected or plasmid-transfected HBMECs exposed to bacteria for various time points as indicated were probed for expression of endothelial cell glycoprotein 96 (Ecgp96), PPAR- $\gamma$ , GLUT-1, and angiotensin II receptor I (AT1R).  $\beta$ -actin was used as a loading control; "C" indicates plasmid-transfected/uninfected control. The blots shown are representative of 2 independent experiments.

HBMECs. Treatment with TS or RG before infection with *E. coli* K1 rescued the glucose uptake levels to those seen in uninfected controls (Figure 3A). In contrast, overexpression of FL-PPAR- $\gamma$  or FL-GLUT-1 enhanced glucose levels despite infection with *E. coli* K1 when compared to levels in HBMECs infected with the bacteria alone (Figure 3B). These observations indicate a direct correlation between PPAR- $\gamma$ , GLUT-1, and glucose uptake and confirm our hypothesis that *E. coli* K1 suppresses glucose uptake mechanisms mediated by PPAR- $\gamma$  and GLUT-1 in HBMECs during infection.

The expression of PPAR- $\gamma$  and GLUT-1 is vital for the barrier integrity of the BBB [24, 30]. We previously reported that disruption of tight junction (TJ) and adherens junction (AJ) integrity plays a key role in *E. coli* K1 translocation across the HBMEC monolayer [27, 31]. Moreover, pretreatment of HBMECs with TS completely inhibited *E. coli* K1-mediated barrier disruption [14]. To determine whether the selective PPAR- $\gamma$  agonist RG or overexpression of PPAR- $\gamma$  and GLUT-1 plasmids also prevented junction disruption, trans-endothelial electrical resistance was measured in *E. coli* K1-infected HBMECs. Similar to TS, RG pretreatment or plasmid overexpression also inhibited the ability of *E. coli* K1 to disrupt barrier integrity (Figure 3C and 3D). Therefore, *E. coli* K1 signals for barrier disruption in HBMECs through the Ecgp96/AT1R/TLR2 complex, and this process involves suppression of PPAR- $\gamma$  and/or GLUT-1.

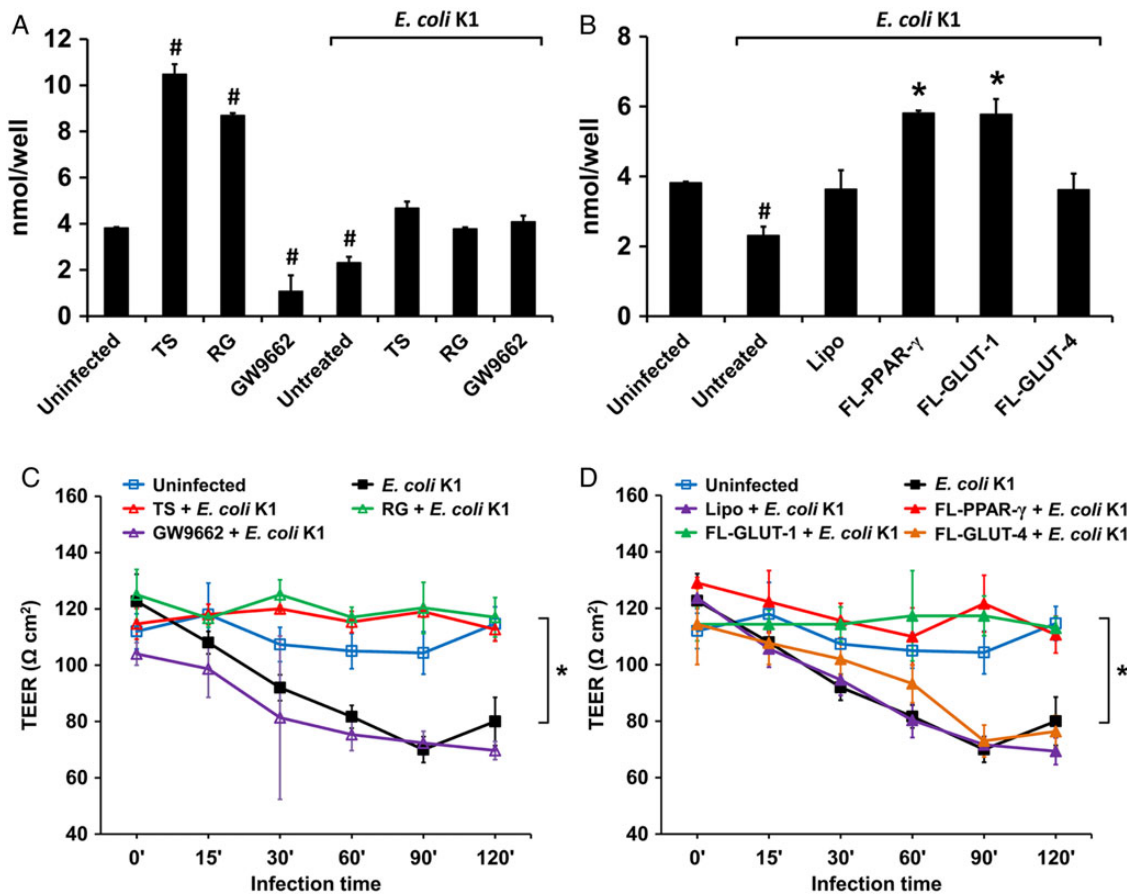
#### Specific Activation of PPAR- $\gamma$ Prevents the Onset of Meningitis in Newborn Mice

Our results show that PPAR- $\gamma$  and GLUT-1 may play a role in modulating glucose uptake in HBMECs, an in vitro model of BBB during *E. coli* K1 invasion. To establish the exclusive role of PPAR- $\gamma$  in a newborn mouse model of *E. coli* K1 meningitis, infection experiments with *E. coli* K1 were performed in 2-day-old mouse pups in the presence or absence of RG pretreatment, as described in Methods. Pretreatment with TS-sulfate (TS-S; a soluble ester of TS) was used as a control, based on our earlier observations that TS-S prevented the onset of meningitis in vivo [14]. Newborn mice infected with *E. coli* K1 were positive for bacteria in the blood, brain, and CSF by 24 hours after infection. In contrast, pups pretreated with TS or RG before infection were resistant to meningitis and survived (Figure 4A–C). Hematoxylin and eosin staining of the brain sections revealed that cortex, meninges, and hippocampus regions of the infected brains were severely affected, while brains from uninfected and compound-pretreated pups showed no such damage (Figure 4D).

#### *E. coli* K1 Alters Proinflammatory Cytokine Profiles in the Serum and Brain of Newborn Pups

*E. coli* K1 infection causes brain damage similar to the human disease in infected mouse pups, as evidenced by pathological damage and neutrophil infiltration [26, 32, 33]. Hematoxylin and eosin staining revealed severe brain pathology in infected pups due to the excessive neutrophil influx caused by proinflammatory cytokines. To identify the pattern of cytokine expression concurrent with disease progression, multiplex cytokine ELISA was performed to check for interleukin 6 (IL-6), interleukin 1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin 12p70 (IL-12p70), interferon  $\gamma$  (IFN- $\gamma$ ), interleukin 4 (IL-4), interleukin 5 (IL-5), and interleukin 10 (IL-10) expression in serum samples and brain homogenates. Results showed that *E. coli* K1 induces proinflammatory IL-6, IL-1 $\beta$ , and TNF- $\alpha$  levels in serum samples (Figure 5A and 5B). No significant changes in IL-12p70, IFN- $\gamma$ , IL-4, IL-5, and IL-10





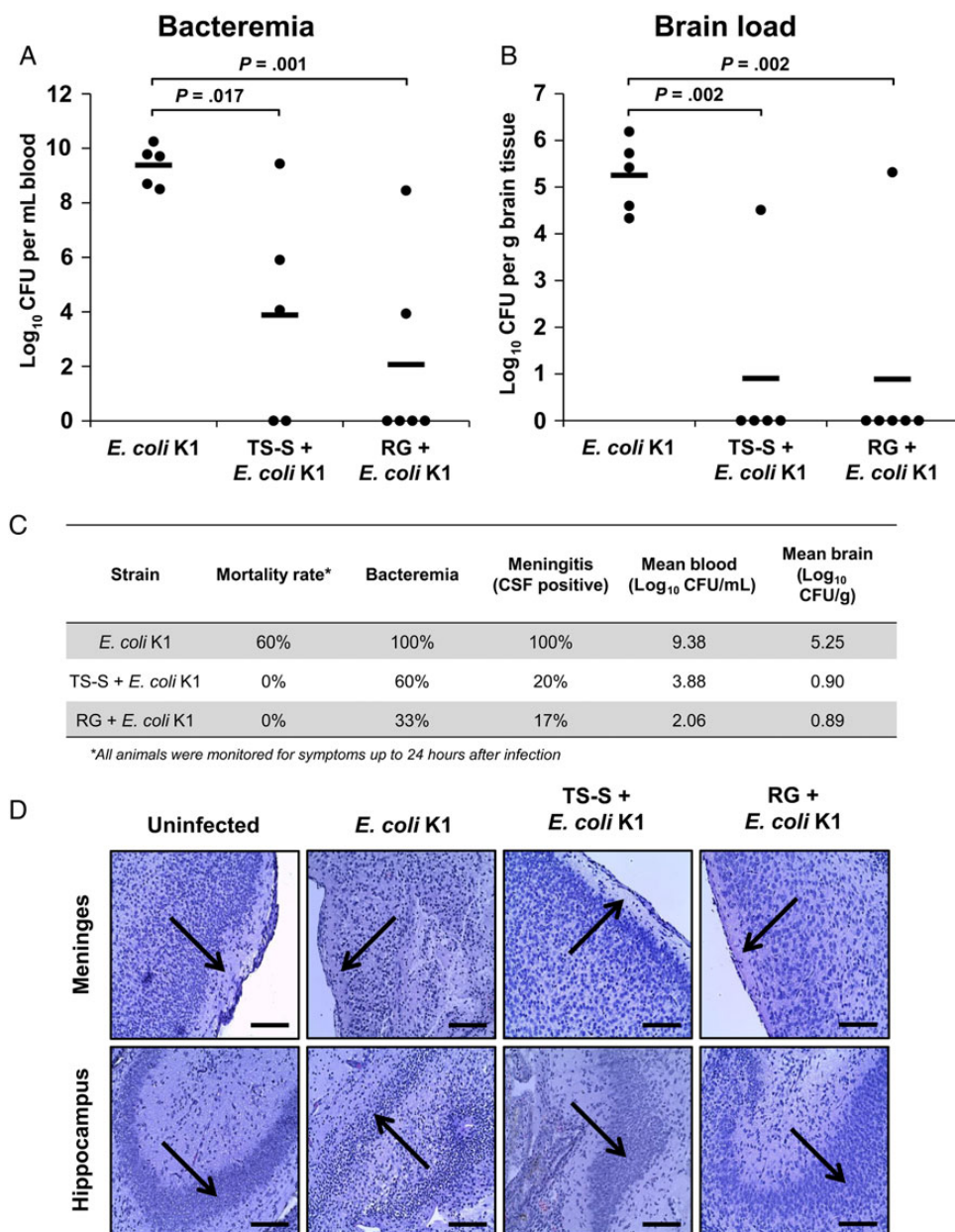
**Figure 3.** *Escherichia coli* K1 suppresses glucose uptake and induces monolayer permeability in human brain microvascular endothelial cells (HBMECs) during the invasion. *A* and *B*, HBMECs were pretreated with compounds (*A*) or transfected with plasmids (*B*) and infected with *E. coli* K1. Colorimetric assessment of glucose uptake was measured using 2-deoxyglucose as substrate. In these graphs, the same results obtained with uninfected HBMECs (uninfected) and *E. coli* K1–infected HBMECs (untreated) were used in both panels for clarity. Error bars indicate standard deviation. #*P* < .05 by the Student *t* test as compared to uninfected cells; \**P* < .05 by the Student *t* test compared to Lipofectamine 3000 (Lipo). *C* and *D*, HBMECs were pretreated with compounds (*C*) or transfected with plasmids (*D*) and infected with *E. coli* K1 for measurement of trans-endothelial electrical resistance (TEER) at various time points. In these graphs, the same results obtained with uninfected HBMECs (uninfected) and *E. coli* K1–infected HBMECs (*E. coli* K1) were used in both TEER experiments for clarity. Error bars indicate standard deviation. \**P* < .05 by the Student *t* test as compared to *E. coli* K1 from 30 to 120 minutes only. Abbreviations: RG, rosiglitazone; TS, telmisartan.

expression were detected. However, only IL-6 was significantly upregulated in the brains of infected pups. Pretreatment with TS or RG inhibited the upregulation of IL-6, IL-1 $\beta$ , and TNF- $\alpha$  levels in the serum. These results show that *E. coli* K1 induces a typical cytokine storm, which primarily involves IL-6, to potentially cause inflammation-mediated damage in the brains of infected mice. Interestingly, TS or RG failed to induce a compensatory mechanism of antiinflammatory cytokine activation (IL-4, IL-5, and IL-10). Rather, the compounds directly suppressed *E. coli* K1–mediated IL-6 upregulation, which may play a role in the eventual clearance of the bacteria from the systemic circulation, thus preventing the onset of meningitis.

#### ***E. coli* K1 Infection Causes Reciprocal Regulation of Ecgp96/AT1R and PPAR- $\gamma$ /GLUT-1 Levels in the Brains of Mouse Pups**

Our *in vitro* data show that *E. coli* K1 suppresses the expression of PPAR- $\gamma$  and GLUT-1 in HBMECs. Therefore, we examined

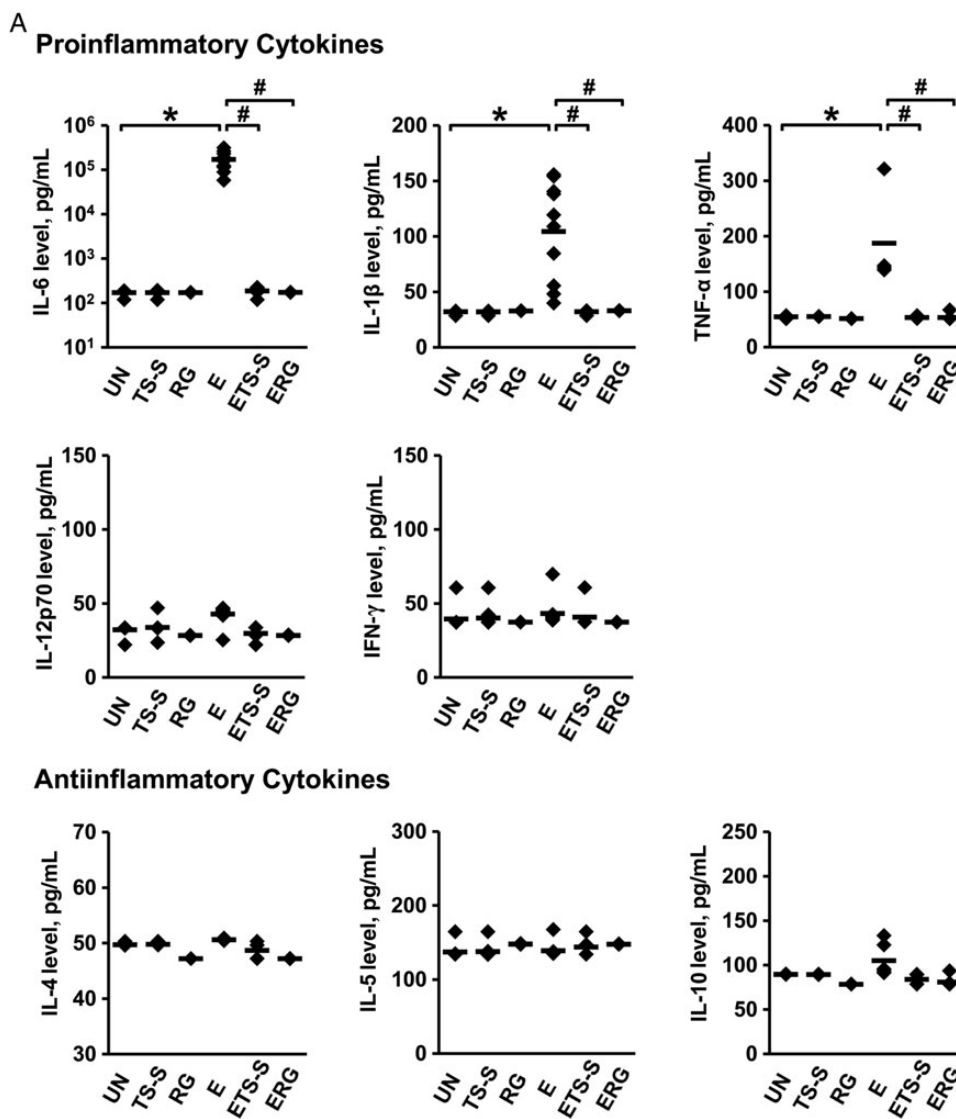
whether *E. coli* K1 infection also causes suppression of these molecules at the BBB *in vivo*. We previously demonstrated that *E. coli* K1 infection affects the cortex, meninges, and hippocampus regions of the brain [9, 26]. Moreover, we showed that Ecgp96 levels are upregulated in these regions of the brain during *E. coli* K1 infection [9]. To determine whether elevated Ecgp96 levels lead to a concomitant upregulation of AT1R and suppression of PPAR- $\gamma$  and GLUT-1 levels, we determined the expression of Ecgp96, AT1R, PPAR- $\gamma$ , and GLUT-1 in brain sections by immunofluorescence. Consistent with previous observations, Ecgp96 levels were elevated in the meninges and hippocampus of infected pups, as were AT1R levels (Figure 6*A* and 6*B*) [9]. However, PPAR- $\gamma$  and GLUT-1 levels were downregulated in the *E. coli* K1–infected brains. In contrast, Ecgp96 and AT1R levels were basal, while PPAR- $\gamma$  and GLUT-1 levels were higher in TS-S and RG + *E. coli* K1 pups than in uninfected pups. Compound alone–treated pups (no



**Figure 4.** Activation of peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) prevents the onset of meningitis in newborn mice. Two-day-old mice were infected with *Escherichia coli* K1 via an intraperitoneal route as described in Methods. *A* and *B*, Blood samples (*A*) and brains (*B*) from infected mice were collected 24 hours after infection, and bacterial load was quantified. Statistical analyses were performed by the Student *t* test (*P* values indicated); analysis of variance verified the significant differences in the means. *C*, Cerebrospinal fluid (CSF) samples were collected and evaluated for the presence of bacteria as described in Methods. Positive CSF cultures were indicative of meningitis. *D*, Brain sections were stained for pathological analysis, using hematoxylin and eosin stain. Arrows indicate normal (uninfected, telmisartan sulfate [TS-S] plus *E. coli* K1, rosiglitazone [RG] plus *E. coli* K1) or damaged (*E. coli* K1) meninges and hippocampi. Scale bar, 100  $\mu$ m.

infection) also showed elevated levels of PPAR- $\gamma$  and GLUT-1 (data not shown), implying that upregulation of their levels by using chemical agonists prevented bacterial translocation across the BBB. To examine the expression of these markers at the BBB, brain microvessels were stained with tomato lectin in the brain sections. Consistent with the patterns above, the expression of Ecgp96 and AT1R was upregulated in the

microvessels of infected tissue, whereas PPAR- $\gamma$  and GLUT-1 levels were downregulated (Figure 6C and 6D). An inverse phenomenon was observed in the brain microvessels of TS-S and RG + *E. coli* K1 pups, compared with *E. coli* K1-infected mouse brains. The localization of Ecgp96, AT1R, and GLUT-1 to the membrane of the microvessels and cytoplasmic expression of PPAR- $\gamma$  are clearly observed. These results suggest that



**Figure 5.** *Escherichia coli* K1 modulates cytokine profiles in newborn mice. Serum samples (A) and brain homogenates (B) were used for cytokine analysis by enzyme-linked immunosorbent assay. Statistical analyses were performed by Student *t* test and analysis of variance. \**P* < .05 by the Student *t* test as compared to uninfected samples (UN); #*P* < .05 by the Student *t* test as compared to *E. coli* K1-infected samples (E). Abbreviations: IFN- $\gamma$ , interferon  $\gamma$ ; IL-1 $\beta$ , interleukin 1 $\beta$ ; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-10, interleukin 10; IL-12p70, interleukin 12p70; RG, rosiglitazone; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; TS-S, telmisartan sulfate.

*E. coli* K1 invasion of brain microvessels downregulates PPAR- $\gamma$  and GLUT-1 expression to infect deeper tissue.

## DISCUSSION

Most *E. coli* strains that cause meningitis contain K1 capsular polysaccharide, which is shown to be necessary for survival in the host [34]. We demonstrated that OmpA is also important for the survival of *E. coli* K1 strains [10]. Mutation in extracellular loops 1 and 2 of OmpA prevented meningitis in newborn mice. These 2 loops have slight differences in their sequences as compared to nonpathogenic *E. coli*. Moreover, they interact with the N-glycans of Ecgp96 to enter HBMECs [9]. Since Ecgp96 upregulation has been correlated with glucose starvation,

we analyzed whether alterations of glucose uptake mechanisms mediated by the OmpA-Ecgp96 interaction play a role in *E. coli* K1 meningitis.

The promotion of glucose uptake by PPAR- $\gamma$  and GLUT-1 has been a well-established paradigm in the maintenance of brain health. [15, 35–37]. However, the role of PPAR- $\gamma$  and/or GLUT-1 in meningitis has not been explored previously. The data presented here using in vitro and in vivo models clearly indicate a critical role played by these markers in the development of neonatal meningitis by *E. coli* K1. We also propose an inverse correlation between Ecgp96 and PPAR- $\gamma$  wherein the binding of *E. coli* K1 OmpA to basal levels of Ecgp96 causes suppression of PPAR- $\gamma$ . This leads to inhibition of glucose uptake due to the

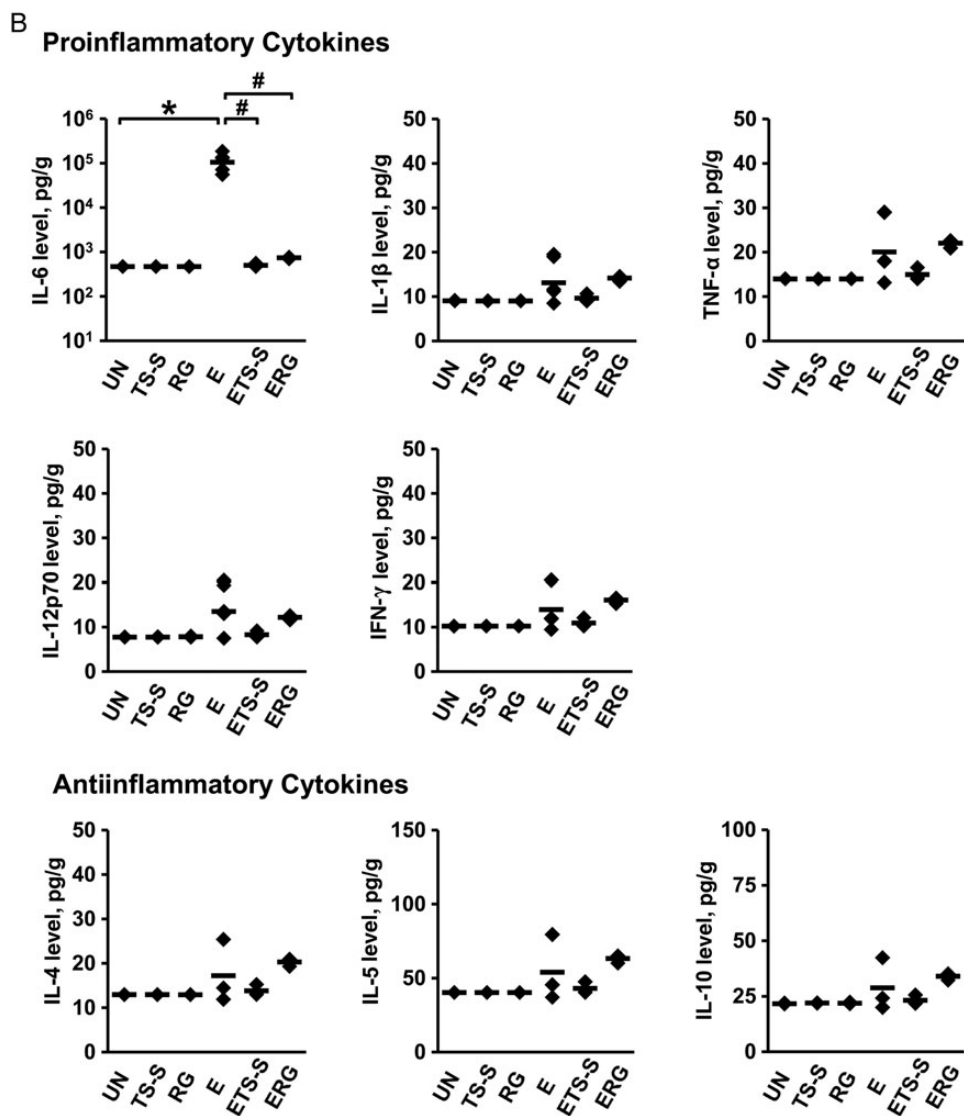


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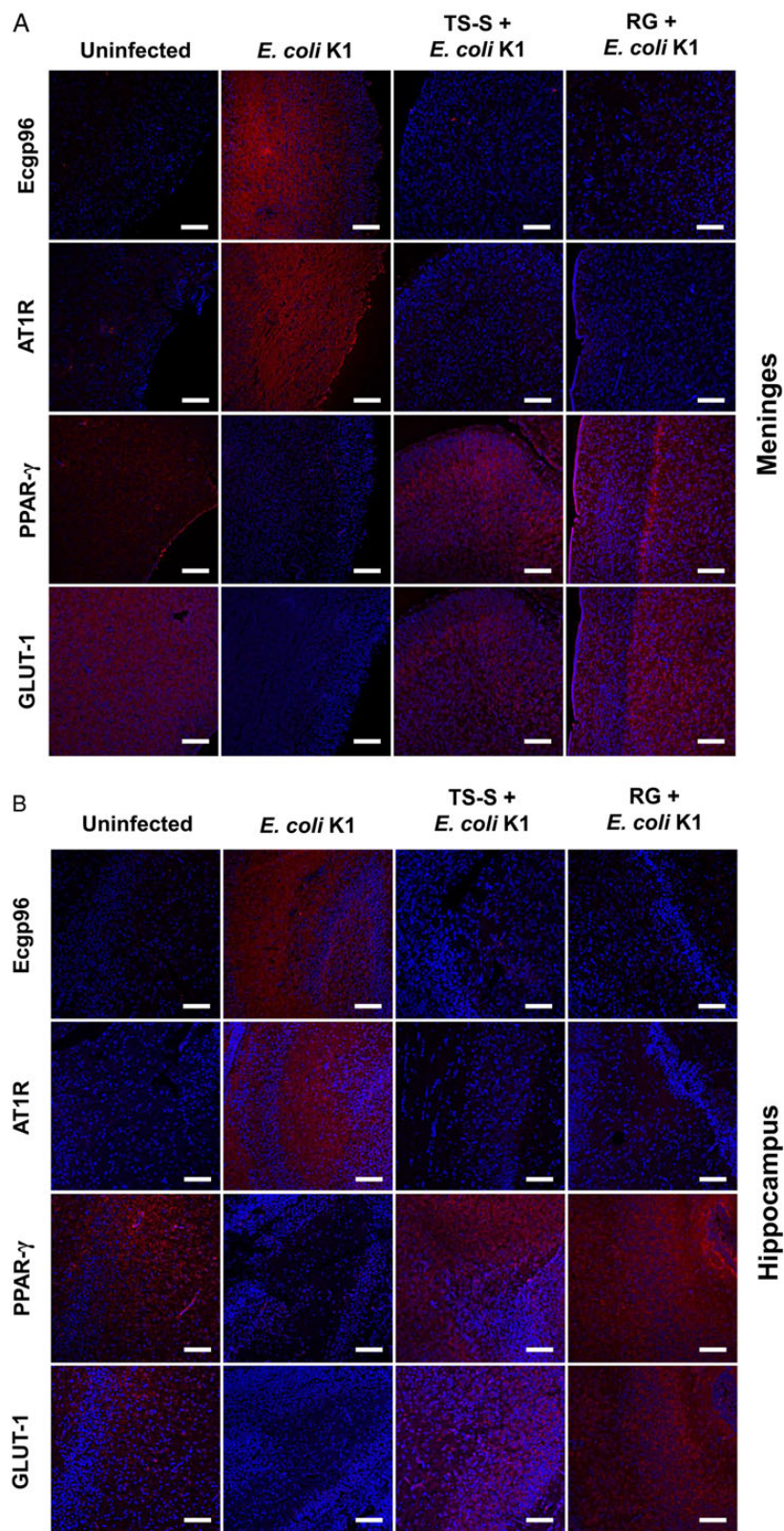
downregulation of GLUT-1 expression. This glucose-deficient environment leads to a stress response in the BBB, thereby further enhancing the expression of Ecgp96 for more bacteria to bind and invade.

Pathogens including *Brucella*, *Listeria*, and enteroaggregative *E. coli* have been recently shown to upregulate PPAR- $\gamma$  [28, 38, 39]. On the contrary, we demonstrated here that *E. coli* K1 suppresses PPAR- $\gamma$  and GLUT-1 levels in the endothelial cells forming the BBB and that upregulation of PPAR- $\gamma$  (using partial and selective PPAR- $\gamma$  agonists) prevented *E. coli* K1 translocation of the BBB to establish infection. The role of PPAR- $\gamma$  and GLUT-1 in the maintenance of TJ and AJ integrity is well recognized [40, 41]. Furthermore, we previously showed that *E. coli* K1 disrupts AJ during the invasion of the BBB [27, 31]. Our current results demonstrated that pharmacological augmentation of

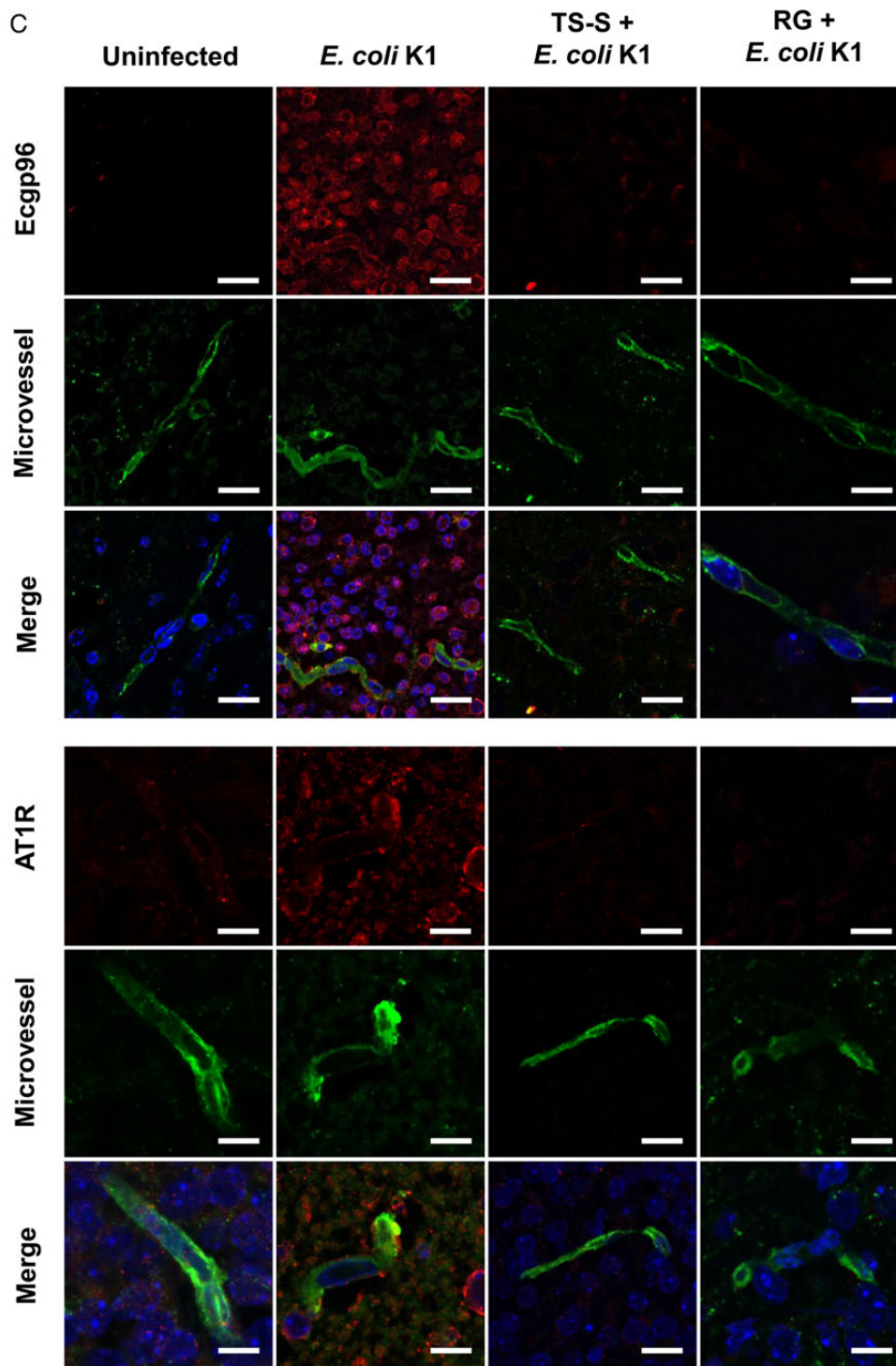
PPAR- $\gamma$  levels by using TS or RG prevents AJ disruption, and therefore manipulation of PPAR- $\gamma$  levels by *E. coli* K1 may be a critical step in the invasion process. We showed that TS pretreatment inhibited *E. coli* K1-mediated AJ disruption [14]. Our current data further confirm that TS exerts its activity as an inhibitor of bacterial invasion by both AT1R antagonism and PPAR- $\gamma$  upregulation. Furthermore, this dual activity of TS was helpful in the establishment of a previously unknown signaling link between *E. coli* K1 OmpA and its receptor Ecgp96 (along with AT1R and TLR2), PPAR- $\gamma$ , and endothelial barrier integrity.

Disruption of BBB integrity causes an influx of inflammatory mediators that cause extensive tissue damage [42]. *E. coli* K1 causes brain damage by inducing neuronal apoptosis and neutrophil infiltration in the brains of infected mouse pups [26].





**Figure 6.** *Escherichia coli* K1 promotes reciprocal regulation of endothelial cell glycoprotein 96 (Ecgp96)/angiotensin II receptor I (AT1R) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ )/glucose transporter 1 (GLUT-1) levels in the brains of newborn mice. The brain sections were stained with antibodies to Ecgp96, AT1R, PPAR- $\gamma$ , and GLUT-1 followed by Alexa 647 fluorophore-coupled (red) secondary antibodies. *A*, and *B*, Meninges (*A*) and hippocampus (*B*) are shown for each treatment. DAPI (blue) was used to stain the nuclei. Scale bar, 100  $\mu$ m. Additionally, the brain sections were stained with tomato lectin (green) to visualize microvessels and for Ecgp96 and AT1R (*C*) and PPAR- $\gamma$  and GLUT-1 (*D*; red) expression in the meninges. DAPI (blue) was used to stain the nuclei. Scale bar, 20  $\mu$ m. This figure is available in black and white in print and in color online.

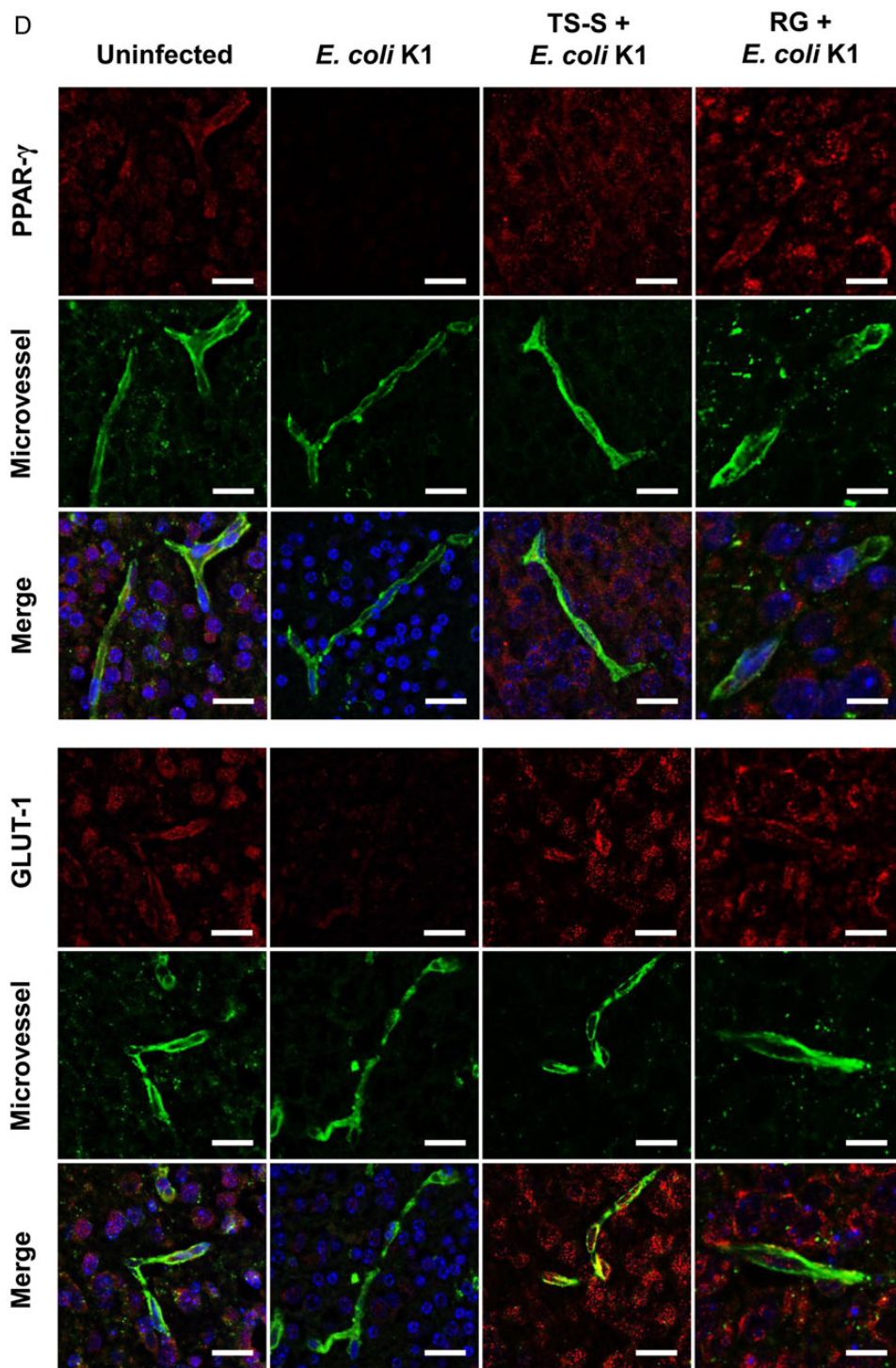


**Figure 6.** *Continued*

However, the specific inflammation patterns underlying the observed pathology in either humans or mice are not well studied. Our results clearly show a prominent role of the proinflammatory cytokine IL-6 as a mediator of brain damage. Interestingly, IL-6 levels were also elevated in meningitis caused by Group B

streptococcus and *Haemophilus influenzae* type B [43, 44]. Moreover, increased IL-6 production by astrocytes and microglia during brain injury or meningitis leads to increased influx of leukocytes into the brain [45, 46]. Therefore, neutrophil infiltration during *E. coli* K1 meningitis may be a result of IL-6





**Figure 6.** *Continued*

production. IL-6 upregulation in the brain corresponding to the lack of PPAR- $\gamma$  activity was recently shown in Alzheimer disease [47]. In agreement, preservation of PPAR- $\gamma$  activity by pretreatment with TS or RG effectively inhibited *E. coli* K1-mediated IL-6 production, promoted bacterial clearance, and

prevented inflammation-related pathology. Another interesting observation here is that although *E. coli* K1-mediated suppression of PPAR- $\gamma$  and GLUT-1 levels is consistent in vitro and in vivo, *E. coli* K1 does not induce cellular damage in HBMECs even >24 hours after infection (unpublished data). Therefore,

the extensive tissue damage seen in vivo maybe the result of IL-6 induction due to lack of PPAR- $\gamma$  activity during infection. However, further studies using IL-6 knockout mice are needed to conclusively demonstrate the role of IL-6 in *E. coli* K1-induced brain damage.

The current study unravels a unique infection strategy used by *E. coli* K1 in the pathogenesis of neonatal meningitis by manipulating PPAR- $\gamma$  and GLUT-1 levels. The modulation of these proteins exhibits a causal effect on barrier disruption and inhibition of glucose uptake, thereby providing new avenues for therapeutic approaches. The inhibition of glucose uptake at the BBB could be an additional factor contributing to the low glucose levels in the CSF during *E. coli* K1 meningitis. The clinical diagnosis of meningitis is based on CSF-positive cultures for *E. coli* K1. However, the study involves pretreatment with PPAR- $\gamma$  agonists before experimentally inducing meningitis. Therefore, the most credible therapeutic strategies would involve the use of antibiotics in conjunction with TS or RG to override suppression of glucose uptake by *E. coli* K1. Our unpublished observations reveal that supplementing antibiotic treatment with TS results in a quicker resolution of brain inflammation in pups compared to those treated and rescued with antibiotics alone. This short-term antibiotic/TS treatment does not seem to have lasting sequelae in these animals when they eventually reach adulthood. Clinical studies indeed show that short-term treatment regimens (ranging from 3 weeks to 24 weeks) with the antihypertensive drug candesartan (an angiotensin receptor blocker similar to TS) in children with hypertension (ages 1–18 years) does not cause any adverse effects [48]. Since brain inflammation during meningitis causes intracranial hypertension, adjunct treatment with TS may also help resolve intracranial hypertension, along with suppression of IL-6 levels, leading to better brain recovery [49]. It is unclear whether the effects of TS or RG on PPAR- $\gamma$  in other cell types contribute to the reduction in blood and brain bacterial levels in *E. coli* K1-infected mice. Further studies using brain endothelial cell-specific PPAR- $\gamma$  knockout mice are needed to validate the role of PPAR- $\gamma$  in the onset of meningitis. Nevertheless, therapeutic intervention with PPAR- $\gamma$  agonists along with antibiotics may eventually evolve into an effective method of preventing morbidity and neurological sequelae in meningitis due to *E. coli* K1 and perhaps other bacterial pathogens.

## Notes

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