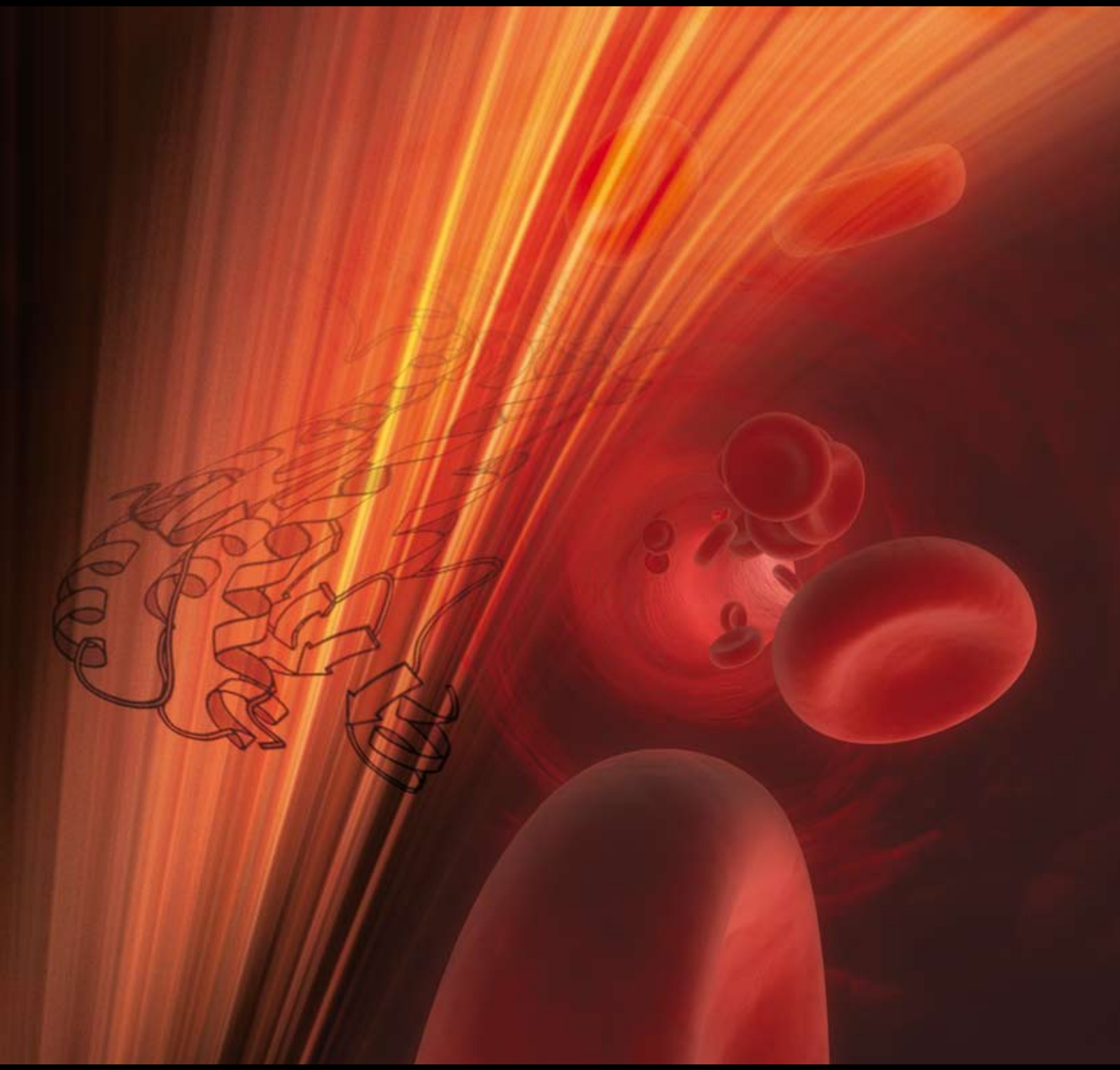
PPAR Research

PPARs in Neuroinflammation

Guest Editor: Michael K. Racke and Paul D. Drew



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Editorial
PPARs in Neuroinflammation

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Welcome to this special issue of PPAR Research dedicated to "PPARs in Neuroinflammation." The central nervous system (CNS) was once thought to be an immune-privileged site void of significant inflammation. However, it is now clear that activated peripheral immune cells are capable or entering and functioning within the CNS. In addition, resident immune cells termed "microglia" protect the CNS through production of molecules which are toxic to pathogens. However, chronically activated microglia can produce molecules toxic to host CNS cells, potentially leading to neurodegeneration. Interestingly, a variety of CNS disorders are characterized by neuroinflammation and associated neurodegeneration. The role of PPARs in modulating lipid and glucose metabolism is well established. More recently, PPARs have been demonstrated to modulate inflammation. For example, PPAR agonists inhibit the production of proinflammatory molecules by peripheral immune cells as well as resident CNS glia. Furthermore, PPAR receptor agonists have proven effective in suppressing the development of animal models of CNS inflammatory and neurodegenerative disorders. This suggests that modulation of PPARs may be effective in treating the related human diseases.

This special issue of PPAR Research contains a series of reviews concerning the role of PPARs in neuroinflammatory diseases. We are fortunate to have received contributions from experts in the fields concerning the potential role of PPARs in modulating CNS disorders including multiple sclerosis, Alzheimer's disease, spinal cord injury, stroke, traumatic brain injury, amyotrophic lateral sclerosis, and Huntington's disease. Also included are reviews concerning the role of PPAR agonists in modulating the function of resident CNS microglia, and the molecular mechanisms by which PPARs regulate inflammatory signaling as related to CNS disease.

We are pleased that this special issue of PPAR Research also contains two original research reports. The first report provides a thorough investigation of the effects of PPAR-γ agonists in modulating the production of proinflammatory molecules by CNS microglia and astrocytes in response to distinct toll-like receptor ligands relevant to infections of the CNS. The second report investigates PPAR-γ agonist effects on amyloid beta-mediated microglial production of cytokines known to alter T-cell differentiation. This study may have important implications concerning the use of amyloid beta immunization for the treatment of Alzheimer's disease.

We hope that you find this special issue of PPAR Research dedicated to PPARs in neuroinflammation to be informative, and that the special issue will generate additional interest in this rapidly evolving field of research.

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Review Article

PPAR Alpha Regulation of the Immune Response and Autoimmune Encephalomyelitis

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PPARs are members of the steroid hormone nuclear receptor superfamily and play an important role in the regulation of lipid metabolism, energy balance, artherosclerosis and glucose control. Recent studies suggest that they play an important role in regulating inflammation. This review will focus on PPAR-α regulation of the immune response. We describe how PPAR-α regulates differentiation of T cells by transactivation and/or interaction with other transcription factors. Moreover, PPAR-α agonists have been shown to ameliorate experimental autoimmune encephalomyelitis (EAE) in mice, suggesting that they could provide a therapy for human autoimmune diseases such as multiple sclerosis.

1. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs)

Peroxisome proliferator-activated receptors (PPARs) are members of the steroid hormone nuclear receptor superfamily. So far, there are three isoforms that have been identified and cloned, including PPAR-α, PPAR-β/δ, and PPAR-γ, and they exhibit different tissue distribution as well as different ligand specificities. PPAR-α is mainly expressed in hepatocytes, cardiac myocytes, and proximal tubular epithelial cells of the kidney. PPAR-γ expression occurs in adipose tissues and colonic mucosal epithelium. PPAR-δ is highly expressed in the placenta and large intestine. They can be activated by polyunsaturated fatty acids [1–4].

PPARs are ligand-activated nuclear receptors and they have been extensively studied in the regulation ofgenes involved in glucose and lipid metabolism. They have been thought to play an important role in the regulation of lipid metabolism, energy balance, inflammation, artherosclerosis and glucose control. Like other nuclear receptor family members, all three members of the PPAR family have distinct functional domains responsible for ligand binding, DNA binding, and coactivator/corepressor binding. They bind to direct repeat 1 (DR1) elements or peroxisome proliferators response elements (PPREs) in the promoter region of target genes and drive the transcription of these target genes [1].

Recent studies have shown that PPARs, including PPAR-α and γ, play a very important role in the regulation of inflammatory responses, through mechanisms involving transactivation or transrepression of gene expression through activation of transcription factors, including NF-κB, AP1, and NFAT. In this review, we will focus on the regulation of PPAR-α on immune responses and their agonists as a potential treatment for autoimmune demyelinating diseases such as multiple sclerosis.

Approximately 350 000 people in the United States have physician-diagnosed multiple sclerosis (MS) [5]. It is the leading cause of neurologic disability in the United States in young adults after trauma, thus most patients suffer from the effects of MS for most of their adult life. The cause of MS remains unknown. An autoimmune process for MS is hypothesized because it shares characteristics of

inflammation and demyelination with its animal model, EAE. Epidemiologic studies and studies examining the disease in identical twins also suggest that both environment and genetics influence expression of the disease and play a role in disease pathogenesis [6]. There are now six drugs approved for use in the treatment of MS by the FDA, however none of these agents are a cure for the disease, so the need for better treatment strategies for MS remains [7–10]. In addition, the unfortunate expression of progressive multifocal leukoencephalopathy (PML) in MS patients treated with nataluzimab highlights the need for medications with a proven safety record [11–13].

Several animal models have been used to study MS. In some of these models, disease is induced by viruses, such as Theiler's virus or Borna disease virus [14]. Of the EAE models, the most commonly studied are those established in the Lewis rat and in several susceptible mouse strains. Murine EAE results in a relapsing-remitting disease, similar to the early phase of disease for most MS patients, whereas EAE in the Lewis rat is a monophasic illness. In chronic murine EAE, the pathology observed in the white matter shows much more demyelination than the Lewis rat model, again being more reminiscent of the pathology seen in the CNS of patients with MS. With the advent of transgenic and homologous recombination technology, it is increasingly clear that many powerful molecular tools are becoming available to study the immune response in pathologic processes such as EAE.

2. REGULATION OF IMMUNE RESPONSES BY PPAR-α

2.1. PPAR-α expression in immune cells

PPAR-α is predominantly expressed in tissues exhibiting high catabolic rates of fatty acids (liver, heart, kidney, and muscle). However, recent studies have shown that it is also expressed in immune cells.

Monocytes and macrophages

Chinetti et al. [15] showed that PPAR-α is expressed in undifferentiated monocytes and in differentiated human monocyte-derived macrophages. PPAR-α is constitutively expressed in the cytoplasm, whereas PPAR-γ is predominantly localized in the nucleus. They both were shown to be transcriptionally active after ligand binding to their receptors. Both PPAR-α and PPAR-γ ligands induce apoptosis of macrophages following activation with tumor necrosis factor-α/interferon-γ.

T and B lymphocytes

PPAR-α has been reported to be expressed in T and B lymphocytes [16, 17]. Jones et al. [16] demonstrated that T and B lymphocytes constitutively express PPAR-α and PPAR-γ. PPAR-α is the predominant isoform expressed in lymphocytes, whereas PPAR-γ dominates in all cell types of the myeloid lineage. However, PPAR-α and PPAR-γ are differentially expressed following lymphocyte activation. PPAR-α expression was downregulated following T-cell activation while PPAR-γ expression increased under the same activating conditions. Exposure to specific ligand determined that PPAR-α in lymphocytes effectively transactivates a peroxisome proliferator response element (PPRE) reporter construct. Ligand activation of lymphocyte PPAR-α antagonized NF-κB. These observations suggested that a functional PPAR-α exists within T cells and B lymphocytes.

Langerhans cells

Epidermal Langerhans cells (LCs) play a pivotal role in initiating and maintaining primary immune responses in the skin. Dubrac et al. [18] showed that PPAR-α is expressed in immature LC and downregulated in mature LC. Pharmacologic PPAR-α activation inhibits LC maturation, migratory capacity, cytokine expression, and the ability to drive T-cell proliferation. Moreover, PPAR-α activation inhibits NF-κB but not stress-activated protein kinase/JNK, p38MAPK, and ERK1/2. This study suggested that PPAR-α activation by endogenous ligands may provide a molecular signal that allows LC to remain in an immature state.

2.2. PPAR-α regulation of inflammation and cytokine production

The study of PPAR-α deficient mice revealed the relationship between PPAR-α and inflammation. Devchand et al. [19] demonstrated that lack of PPAR-α activity increases inflammatory responses. They showed that inflammation due to inflammatory agents, including arachidonic acid and LTB4, is prolonged in PPAR-α deficient mice as compared to wild-type mice. The β and γ PPAR subtypes did not compensate for a lack of PPAR-α in an LTB4-mediated inflammatory response.

Delerive et al. [20] showed another possible mechanism of PPAR-α regulating inflammation. They demonstrated that PPAR-α negatively regulates the vascular inflammatory gene response by negative cross-talk with the transcription factors NF-κB and AP-1. They showed that aortic explants isolated from PPAR-α-null mice display an exacerbated response to inflammatory stimuli, such as lipopolysaccharide (LPS), as demonstrated by increased IL-6 secretion.

Cytokines are one of the major factors directing T-cell differentiation and play an important role in the pathogenesis of autoimmune diseases. Recent studies have shown that PPAR-α regulates the expression of cytokines which are critical in autoimmune disease (see below). Splenocytes harvested from PPAR-α agonist, WY14,643, fed and pMOG(35–55) immunized mice showed impaired production of IFN-γ, IL-6, and TNF-α despite similar proliferative responses, following in vitro restimulation with pMOG(35–55). It was also observed that IL-4 expression in cultures of mitogen-activated splenocytes was increased [21].

Lee et al. [22] reported that the PPAR-α agonist, Fenofibrate, repressed IL-17 and interferon-gamma expression and improved colitis in IL-10-deficient mice. PPAR-α was found to be expressed in lymphocytes, macrophages, and crypt and surface epithelial cells of the colon. Colonic expression

of interferon-gamma and IL-17 genes was decreased in IL-10 deficient mice, when the mice were treated with fenofibrate. Fenofibrate also repressed interferon-gamma and IL-17 expression in isolated T cells, the expression of the genes encoding the chemokines, CXCL10, CCL2, and CCL20, and repressed CXCL10 gene promoter activity in tumor necrosis factor-alpha-treated HT-29 cells.

Jones et al. [23] reported that unliganded PPAR-α suppressed T-bet expression and decreased IFN-γ production in T cells. They demonstrated that activated CD4(+) T cells lacking PPAR-α produce increased levels of IFN-γ, but significantly lower levels of IL-2 when compared with activated wild-type CD4(+) T cells.

Another study by Dasgupta et al. [24] suggested that PPAR-α increased the activity of GATA-3 and inhibited expression of T-bet, which would be in agreement with prior studies which showed that PPAR-α agonists increased IL-4 production by T cells. Interestingly, this study also suggested that the PPAR-α agonist gemfibrozil could inhibit clinical signs of EAE in mice deficient in PPAR-α, with concomitant upregulation of IL-4 and inhibition of IFN-γ [24]. This study did not indicate whether the same changes in T-bet and GATA-3 expression also occurred in PPAR-α deficient mice.

Delerive et al. [20] showed fibrate treatment represses IL-6 mRNA levels in LPS-stimulated aortas of PPAR-α wild-type, but not of PPAR-α-null mice, demonstrating a role for PPAR-α in this fibrate action. In human aortic smooth muscle cells, fibrates inhibit IL-1-induced IL-6 gene expression.

2.3. Possible mechanisms

Like other transcription factors, PPARs are able to positively regulate gene expression by binding to PPRE as a heterodimer with the retinoic acid X receptor (RXR). In the unliganded state, PPARs are associated with a nuclearreceptor corepressor. In addition, heat shock protein-90 and the hepatitis virus B X-associated protein 2 have been shown to associate with PPAR-α and negatively regulate subsequent gene activation [25, 26]. Upon activation, the PPARs undergo a conformational change that results in the dissociation from the corepressor, enabling the PPAR to bind nuclear receptor coactivators. These coactivators then act to reorganize the chromatin templates allowing the basal transcription machinery to gain access to the promoter regions driving transcription of target genes.

In our lab, we have investigated the mechanism by which the PPAR-α agonist gemfibrozil induces immune deviation and protects mice from EAE. Similar to the studies by Dasgupta [24], we demonstrated that treatment with gemfibrozil increases GATA-3 and decreases T-bet expression in vitro and directly ex-vivo. These changes correlated with an increase in nuclear PPAR-α expression. Moreover, the protective effects of gemfibrozil in EAE were shown to be partially dependent on IL-4 and to occur in a receptor-dependent manner. PPAR-α was shown to regulate the IL-4 and IL-5 genes and bound the IL-4 promoter in the presence of steroid receptor coactivator-1 (SRC-1), suggesting transactivation of the IL-4 gene (Figure 1) [27].

PPARs cannot only induce but also repress gene transcription. One recent study showed a sumoylation-dependent pathway mediating transrepression of inflammatory response genes by PPAR-γ in macrophages [28]. The initial step of this pathway involves ligand-dependent sumoylation of the PPAR-γ ligand-binding domain, which targets PPAR-γ to nuclear receptorcorepressor (NCoR)-histone deacetylase-3 (HDAC3) complexes on inflammatory gene promoters. This in turn prevents recruitment of the ubiquitylation/19S proteasome machinery that normally mediates the signal-dependent removal of corepressor complexes required for gene activation. As a result, NCoR complexes are not cleared from the promoter and target genes are maintained in a repressed state. This mechanism provides an explanation for how an agonist-bound nuclear receptor can be converted from an activator of transcription to a promoter-specific repressor of NF-κB target genes. However, so far there is no evidence showing that PPAR-α is able to repress target genes by this sumoylation-dependent pathway.

Activated PPAR-α has been demonstrated to exert anti-inflammatory activities through its ability to antagonize other signaling pathways, in part through the interaction with other transcription factors, including NF-κB, AP-1, and STATs (see below).

Spencer et al. [29] have demonstrated that therapeutic treatment of aged mice with PPAR-α activating agents corrected abnormal nuclear NF-κB activity, redu ced lipid peroxide levels, and eliminated the dysregulated expression of cytokines and other genes under NF-κB control.

Delerive et al. showed activation of PPAR-α represses both c-Jun- and p65-induced transcription of the human IL-6 promoter. Glutathione S-transferase (GST) pull-down experiments demonstrated that PPAR-α physically interacts with c-Jun, p65, and CBP [20]. They further showed that fibrates, synthetic PPAR-α activators, induced the expression of the inhibitory protein IκBα in human aortic smooth muscle cells as well as in primary human hepatocytes. They demonstrated that fibrates induced IκBα in liver in vivo and that this action required PPAR-α. Furthermore, fibrate treatment induced IκBα protein expression in the cytoplasm and also enhanced IL-1β-induced accumulation of IκBα protein in the nucleus [30]. These actions of fibrates on IκBα expression were accompanied by a decrease in NF-κB DNA binding activity. They further demonstrated that induction of IκBα gene transcription by PPAR-α is DNA binding-independent. They demonstrated that PPAR-α potentiates p65-stimulated IκBα transcription in a ligand-dependent manner. PPAR-α activation of IκBα transcription requires the NF-κB and Sp1 sites within the IκBα promoter. PPAR-α activation enhances the occupancy of the NF-κB response element in IκBα promoter in vivo. VDR-interacting protein 205 (DRIP205) is required to regulate IκBα promoter activity [31].

PPAR-α was also found to negatively regulate the transcription of T-bet. T-bet is a key regulator of the IFNγ gene in Th1 cells. The induction of T-bet expression in CD4(+) T cells was determined to be positively influenced by p38 mitogen-activated protein (MAP) kinase activation, and

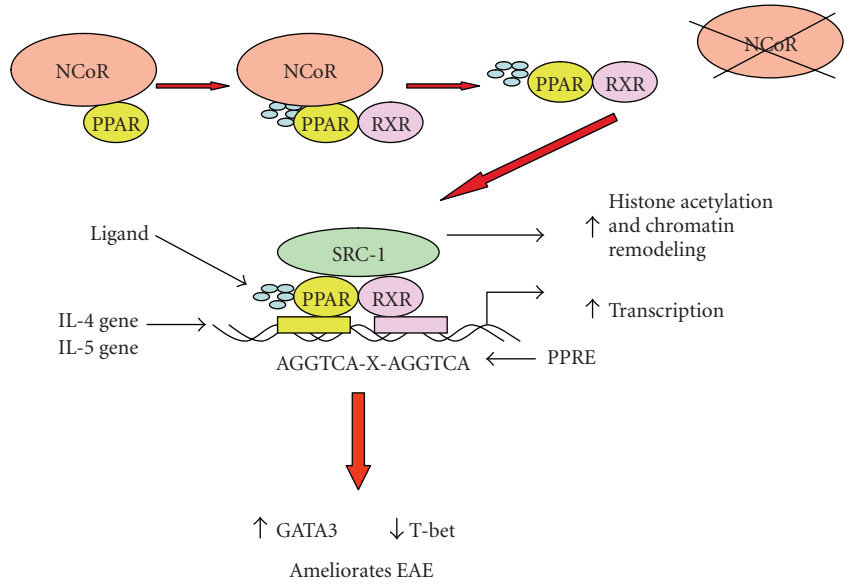

Figure 1: *A model for PPAR-α-mediated protection in EAE*. In the presence of PPAR-α agonists, PPAR-α heterodimerizes with RXR, dissociates from its nuclear corepressor complex, associates with a coactivator complex, and binds to PPREs in the promoter region of IL-4 and/or IL-5. The transactivation of IL-4/IL-5 leads to increased expression of GATA-3 which in turn results in decreased T-bet expression and downregulation of the Th1/Th17 inflammatory response. This shift in the immune response to a Th2-like phenotype results in amelioration of EAE.

the presence of unliganded PPAR-α effectively suppressed the phosphorylation of p38 MAP kinase. The activation of PPAR-α with highly specific ligands relaxed its capacity to suppress p38 MAP kinase phosphorylation and promoted T-bet expression [23]. This observation conflicts with the observation of Dasgupta [24] and our own work.

Lee et al. found that four PPAR-α activators suppressed lipopolysaccharide-stimulated STAT1 phosphorylation and nuclear factor binding to γ-interferon-activated sequence/interferon-α-stimulated response element sites known to contain STAT binding sites. PPAR-α activators also suppressed lipopolysaccharide-stimulated tumor necrosis factor-α and monocyte chemoattractant protein-1 transcription and release [32].

In addition to PPAR-α dependent transcriptional regulation, Selim et al. [33] showed that fibrates upregulate TRB3 in lymphocytes independent of PPAR-α by augmenting CCAAT/enhancer-binding protein β (C/EBP-β) expression. They demonstrated that fibrates upregulate TRB3 expression (a protein that interferes with insulin-induced activation of AKT), in mitogen-activated lymphocytes of both wild type and knockout mice, suggesting that upregulation of this protein occurs in a PPAR-α-independent manner.

Dasgupta et al. [24] showed gemfibrozil inhibited the encephalitogenicity of MBP-primed T cells and switched the immune response from a Th1 to a Th2 profile independent of PPAR-α. Gemfibrozil consistently inhibited the expression and DNA-binding activity of T-bet, and stimulated the expression and DNA-binding activity of GATA-3, a key regulator of IL-4. Gemfibrozil treatment decreased the number of T-bet-positive T cells and increased the number of GATA-3-positive T cells in the spleens of donor mice. Gemfibrozil was shown to have an inhibitory effect on the invasion of T-bet-positive T cells into the spinal cord of EAE mice. Furthermore, they demonstrate that the differential effect of gemfibrozil on the expression of T-bet and GATA-3 was due to its inhibitory effect on NO production.

3. PPAR ALPHA AND AUTOIMMUNE ENCEPHALOMYELITIS

Organ-specific autoimmune diseases, such as multiple sclerosis (MS) and its animal model, are mediated by IFN-γ and/or IL-17 producing CD4 T helper cells. Since PPAR-α regulates inflammation and cytokine production, PPAR-α agonists have been tested as a potential treatment for autoimmune diseases.

Lovett-Racke et al. [34] demonstrated that PPAR-α agonists can be used as a therapy for autoimmune disease. They demonstrated that PPAR-α agonists can increase the production of the Th2 cytokine, IL-4, and suppress proliferation by TCR transgenic T cells specific for the myelin basic protein Ac1-11, as well as reduce NO production by microglia. Oral administration of gemfibrozil and fenofibrate inhibited clinical signs of experimental autoimmune encephalomyelitis. More importantly, gemfibrozil was shown to shift the cytokine secretion of human T-cell lines by inhibiting IFN-γ and promoting IL-4 secretion. These results suggest that PPAR-α agonists, such as gemfibrozil and fenofibrate, may be attractive candidates for use in human inflammatory conditions such as multiple sclerosis.

In another study, Dasgupta et al. [24] demonstrated that gemfibrozil ameliorates relapsing-remitting EAE independent of PPAR-α. They showed that clinical signs of EAE,

infiltration of mononuclear cells, and demyelination were significantly lower in mice receiving gemfibrozil, suggesting gemfibrozil may find therapeutic use in multiple sclerosis.

Interestingly, PPAR-α expression in T cells was suggested to mediate gender differences in development of T-cell-mediated autoimmunity. Dunn et al. [35] showed that PPAR-α is more abundant in male as compared with female CD4(+) cells and that its expression is sensitive to androgen levels. Upon induction of EAE, male PPAR-α $(-/-)$ mice developed more severe clinical signs than females. These results suggest that males are less prone to develop Th1-mediated autoimmunity because they have higher T-cell expression of PPAR-α.

Xu et al. [36] investigated the effects of PPAR-α agonists on primary mouse microglia, a cell type implicated in the pathology of MS and EAE. They demonstrated that PPAR-α agonists inhibited the secretion of IL-1β, TNF-α, IL-6, and IL-12 p40 and the chemokine MCP-1 by LPS-stimulated microglia. Retinoid X receptors (RXRs) physically interact with PPAR-α receptors, and the resulting heterodimers regulate the expression of PPAR-responsive genes. They demonstrated that the PPAR-α agonists ciprofibrate, fenofibrate, gemfibrozil, and WY14,643 inhibited NO production by stimulated microglia in a dose-dependent manner. Furthermore, a combination of 9-cis RA and the PPAR-α agonist fenofibrate cooperatively inhibited NO production by these cells. This study suggested that PPAR-α and RXR agonists might have benefit as a therapy in MS, where activated microglia are believed to contribute to disease pathology.

Other than microglia cells, they also investigated the effects of PPAR-α agonists on primary mouse astrocytes [37]. They observed similar inhibition on cytokine production by PPAR-α agonists. PPAR-α agonists inhibited the secretion of TNF-α, IL-1β, and IL-6 by LPS-stimulated astrocytes. Additionally, fenofibrate inhibited NF-κB DNA binding activity, suggesting a mechanism by which PPAR-α agonists may regulate the expression of genes encoding these proinflammatory molecules. Retinoid X receptors (RXRs) physically interact with PPAR-α receptors, and the resulting heterodimers regulate the expression of PPAR-responsive genes.

They further demonstrated that fenofibrate suppression of EAE was associated with decreased expression of IL-12 family cytokine mRNAs as well as mRNAs encoding TLR4, CD14, and MyD88. They showed that the PPAR-α agonist fenofibrate inhibited the secretion of IL-12p40, IL-12p70 (p35/p40), IL-23 (p19/p40), and IL-27p28 by lipopolysaccharide-stimulated microglia. Furthermore, fenofibrate inhibited microglial expression of CD14 which plays a critical role in TLR signaling [38].

4. CONCLUSION

The functional expression of PPAR-α by several immune cell types suggests that this receptor may play a very important role in regulation of immune responses. Recent studies demonstrate that PPAR-α regulates different aspects of immune responses, including inflammation and cytokine production. Moreover, several studies showed evidence that PPAR-α agonists have potent effects in regulating immune responses and ameliorating EAE. However, the detailed mechanisms have not been completely delineated. Better understanding of the molecular mechanism by which PPAR-α regulates cytokine pathways in immune cells will be very helpful for further development of PPAR-α agonists as a therapy for autoimmune diseases.

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REFERENCES

[1] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[2] D. J. Mangelsdorf, C. Thummel, M. Beato, et al., "The nuclear receptor super-family: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[3] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[4] S. A. Kliewer, B. M. Forman, B. Blumberg, et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

[5] D. W. Anderson, J. H. Ellenberg, C. M. Leventhal, S. C. Reingold, M. Rodriguez, and D. H. Silberberg, "Revised estimate of the prevalence of multiple sclerosis in the United States," *Annals of Neurology*, vol. 31, no. 3, pp. 333–336, 1992.

[6] A. D. Sadovnick and G. C. Ebers, "Epidemiology of multiple sclerosis: a critical overview," *Canadian Journal of Neurological Sciences*, vol. 20, no. 1, pp. 17–29, 1993.

[7] The IFNB Multiple Sclerosis Study Group, "Interferon beta-1b is effective in relapsing-remitting multiple sclerosis—I: clinical results of a multicenter, randomized, double-blind, placebo-controlled trial," *Neurology*, vol. 43, no. 4, pp. 655–661, 1993.

[8] L. D. Jacobs, D. L. Cookfair, R. A. Rudick, et al., "Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis," *Annals of Neurology*, vol. 39, no. 3, pp. 285–294, 1996.

[9] K. P. Johnson, B. R. Brooks, J. A. Cohen, et al., "Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind, placebo-controlled trial," *Neurology*, vol. 45, no. 7, pp. 1268–1276, 1995.

[10] E. Millefiorini, C. Gasperini, C. Pozzilli, et al., "Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome," *Journal of Neurology*, vol. 244, no. 3, pp. 153–159, 1997.

[11] B. K. Kleinschmidt-DeMasters and K. L. Tyler, "Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis," *The*

New England Journal of Medicine, vol. 353, no. 4, pp. 369–374, 2005.

[12] A. Langer-Gould, S. W. Atlas, A. J. Green, A. W. Bollen, and D. Pelletier, "Progressive multifocal leukoencephalopathy in a patient treated with natalizumab," *The New England Journal of Medicine*, vol. 353, no. 4, pp. 375–381, 2005.

[13] G. Van Assche, M. Van Ranst, R. Sciot, et al., "Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease," *The New England Journal of Medicine*, vol. 353, no. 4, pp. 362–368, 2005.

[14] S. D. Miller and W. J. Karpus, "The immunopathogenesis and regulation of T-cell-mediated demyelinating diseases," *Immunology Today*, vol. 15, no. 8, pp. 356–361, 1994.

[15] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *The Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[16] D. C. Jones, X. Ding, and R. A. Daynes, "Nuclear receptor peroxisome proliferator-activated receptor α (PPARα) is expressed in resting murine lymphocytes. The PPARα in T and B lymphocytes is both transactivation and transrepression competent," *The Journal of Biological Chemistry*, vol. 277, no. 9, pp. 6838–6845, 2002.

[17] R. Cunard, M. Ricote, D. DiCampli, et al., "Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors," *Journal of Immunology*, vol. 168, no. 6, pp. 2795–2802, 2002.

[18] S. Dubrac, P. Stoitzner, D. Pirkebner, et al., "Peroxisome proliferator-activated receptor-α activation inhibits Langerhans cell function," *Journal of Immunology*, vol. 178, no. 7, pp. 4362–4372, 2007.

[19] P. R. Devchand, H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli, "The PPARα-leukotriene B_4 pathway to inflammation control," *Nature*, vol. 384, no. 6604, pp. 39–43, 1996.

[20] P. Delerive, K. De Bosscher, S. Besnard, et al., "Peroxisome proliferator-activated receptor α negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1," *The Journal of Biological Chemistry*, vol. 274, no. 45, pp. 32048–32054, 1999.

[21] R. Cunard, D. DiCampli, D. C. Archer, et al., "WY14,643, a PPARα ligand, has profound effects on immune responses in vivo," *Journal of Immunology*, vol. 169, no. 12, pp. 6806–6812, 2002.

[22] J. W. Lee, P. J. Bajwa, M. J. Carson, et al., "Fenofibrate represses interleukin-17 and interferon-γ expression and improves colitis in interleukin-10-deficient mice," *Gastroenterology*, vol. 133, no. 1, pp. 108–123, 2007.

[23] D. C. Jones, X. Ding, T. Y. Zhang, and R. A. Daynes, "Peroxisome proliferator-activated receptor α negatively regulates T-bet transcription through suppression of p38 mitogen-activated protein kinase activation," *Journal of Immunology*, vol. 171, no. 1, pp. 196–203, 2003.

[24] S. Dasgupta, A. Roy, M. Jana, D. M. Hartley, and K. Pahan, "Gemfibrozil ameliorates relapsing-remitting experimental autoimmune encephalomyelitis independent of peroxisome proliferator-activated receptor-α," *Molecular Pharmacology*, vol. 72, no. 4, pp. 934–946, 2007.

[25] W. K. Sumanasekera, E. S. Tien, R. Turpey, J. P. Vanden Heuvel, and G. H. Perdew, "Evidence that peroxisome proliferator-activated receptor α is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2," *The Journal of Biological Chemistry*, vol. 278, no. 7, pp. 4467–4473, 2003.

[26] W. K. Sumanasekera, E. S. Tien, J. W. Davis II, R. Turpey, G. H. Perdew, and J. P. Vanden Heuvel, "Heat shock protein-90 (Hsp90) acts as a repressor of peroxisome proliferator-activated receptor-α (PPARα) and PPARβ activity," *Biochemistry*, vol. 42, no. 36, pp. 10726–10735, 2003.

[27] A. Gocke, R. Hussain, L. Ben, P. D. Drew, A. E. Lovett-Racke, and M. K. Racke, "Transcriptional modulation of the immune response by peroxisomal proliferator-activated receptor-α agonists in autoimmune disease," submitted.

[28] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[29] N. F. L. Spencer, M. E. Poynter, S.-Y. Im, and R. A. Daynes, "Constitutive activation of NF-κB in an animal model of aging," *International Immunology*, vol. 9, no. 10, pp. 1581–1588, 1997.

[30] P. Delerive, P. Gervois, J.-C. Fruchart, and B. Staels, "Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators," *The Journal of Biological Chemistry*, vol. 275, no. 47, pp. 36703–36707, 2000.

[31] P. Delerive, K. De Bosscher, W. V. Berghe, J.-C. Fruchart, G. Haegeman, and B. Staels, "DNA binding-independent induction of IκBα gene transcription by PPARα," *Molecular Endocrinology*, vol. 16, no. 5, pp. 1029–1039, 2002.

[32] J. H. Lee, E. H. Joe, and I. Jou, "PPAR-alpha activators suppress STAT1 inflammatory signaling in lipopolysaccharide-activated rat glia," *NeuroReport*, vol. 16, no. 8, pp. 829–833, 2005.

[33] E. Selim, J. T. Frkanec, and R. Cunard, "Fibrates upregulate TRB3 in lymphocytes independent of PPARα by augmenting CCAAT/enhancer-binding proteinβ (C/EBPβ) expression," *Molecular Immunology*, vol. 44, no. 6, pp. 1218–1229, 2007.

[34] A. E. Lovett-Racke, R. Z. Hussain, S. Northrop, et al., "Peroxisome proliferator-activated receptor α agonists as therapy for autoimmune disease," *Journal of Immunology*, vol. 172, no. 9, pp. 5790–5798, 2004.

[35] S. E. Dunn, S. S. Ousman, R. A. Sobel, et al., "Peroxisome proliferator-activated receptor (PPAR)α expression in T cells mediates gender differences in development of T cell-mediated autoimmunity," *Journal of Experimental Medicine*, vol. 204, no. 2, pp. 321–330, 2007.

[36] J. Xu, P. D. Storer, J. A. Chavis, M. K. Racke, and P. D. Drew, "Agonists for the peroxisome proliferator-activated receptor-α and the retinoid X receptor inhibit inflammatory responses of microglia," *Journal of Neuroscience Research*, vol. 81, no. 3, pp. 403–411, 2005.

[37] J. Xu, J. A. Chavis, M. K. Racke, and P. D. Drew, "Peroxisome proliferator-activated receptor-α and retinoid X receptor agonists inhibit inflammatory responses of astrocytes," *Journal of Neuroimmunology*, vol. 176, no. 1-2, pp. 95–105, 2006.

[38] J. Xu, M. K. Racke, and P. D. Drew, "Peroxisome proliferator-activated receptor-α agonist fenofibrate regulates IL-12 family cytokine expression in the CNS: relevance to multiple sclerosis," *Journal of Neurochemistry*, vol. 103, no. 5, pp. 1801–1810, 2007.

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Review Article

PPAR-γ: Therapeutic Potential for Multiple Sclerosis

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The role of peroxisome proliferator-activated receptors (PPARs) in altering lipid and glucose metabolism is well established. More recent studies indicate that PPARs also play critical roles in controlling immune responses. We and others have previously demonstrated that PPAR-γ agonists modulate the development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). This review will discuss the cellular and molecular mechanisms by which these agonists are believed to modulate disease. The therapeutic potential of PPAR-γ agonists in the treatment of multiple sclerosis will also be considered.

1. INTRODUCTION

Multiple sclerosis (MS) is the second most common neurologic disorder of young adults, behind neurotrauma. Approximately 350000–400000 individuals have physician diagnosed MS in the United States alone. MS is commonly diagnosed around the third decade of life and many patients suffer the devastating effects of the disease for much of their adult lives. The etiology of MS is not completely understood but is believed to result from a combination of genetic and environmental factors. The disease is characterized by inflammation of the central nervous system (CNS), demyelination, and either relapsing-remitting or progressive clinical presentations. Similarities to experimental autoimmune encephalomyelitis (EAE), an established animal model of MS which is elicited following generation and attack of autoreactive T cells against brain tissues suggests an autoimmune origin for MS. In addition to autoreactive T cells, other peripheral immune cells including B cells, monocytes, and dendritic cells may play a role in the pathogenesis associated with MS. In addition, resident CNS cells including chronically activated glial cells are believed to play a role in disease pathogenesis [1].

Nuclear receptors are a family of transcription factors that regulate gene expression in response to ligand binding. Nuclear receptor superfamily members include peroxisome proliferator-activated receptors (PPARs) as well as androgen, estrogens, progesterone, thyroid, and glucocorticoid receptors. Additional orphan nuclear receptors exist for which ligands have not been identified. The critical role of PPARs in modulating glucose and lipid metabolism has been extensively documented [2]. More recently, a role for PPARs in altering immune responses has been established. A role for PPARs in modulation of immune responses was suggested by the observation that indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) binds PPAR-γ. Furthermore, it was documented that PPAR-γ is expressed by cells of the monocyte/macrophage lineage. These observations led to seminal studies demonstrating that PPAR-γ agonists suppress the activation of monocyte/macrophages [3, 4]. Three PPAR isoforms, PPAR-α, -β/δ, and -γ, have been identified. These receptors exhibit distinct tissue expression patterns and ligand specificities [2, 5]. Eicosanoids, polyunsaturated fatty acids, and the cyclopentenone prostaglandin 15d-PGJ_2 are naturally occurring PPAR-γ ligands. Synthetic PPAR-γ ligands include thiazolidinediones which are used for the treatment of type II diabetes.

As transcription factors, PPARs primarily function to regulate the expression of specific genes. Similar to other nuclear receptors, PPARs bind DNA and regulate gene expression as dimers. PPARs form heterodimers with

retinoid-X-receptors (RXRs), and bind DNA at conserved *peroxisome-proliferator response elements* (PPREs) present in the promoter of PPAR-responsive target genes. Upon ligand binding, the PPAR/RXR heterodimer associates with coactivator complexes, binds PPREs, and activates the transcription of PPAR-responsive genes. In contrast, PPAR/RXR heterodimers not bound by ligand associate with corepressor complexes resulting in suppression of gene transcription [2]. PPAR ligands principally activate transcription of genes encoding proteins important in lipid and glucose metabolism by triggering PPAR/RXR binding to PPREs present in the promoters of these genes. In contrast, PPAR agonists generally suppress the expression of genes encoding proinflammatory molecules through a mechanism not involving PPAR/RXR binding to PPREs. This mechanism, termed receptor-dependent transrepression, is believed to occur through physical interaction between PPAR/RXR and other transcription factors which normally activate transcription of proinflammatory genes. Physical interaction with PPAR/RXR inhibits binding of these transcription factors to response elements present on genes encoding proinflammatory molecules, thus suppressing the activation of these genes. Receptor-dependent transrepression may also result from PPAR/RXR interaction with transcriptional coactivator or corepressor molecules that are in limited supply, or PPAR/RXR interactions with the basal transcription machinery [6, 7]. PPAR-γ agonists inhibit transcription factors including NF-κB, AP-1, and STAT-1 from activating gene expression through receptor-dependent transrepression [8]. The mechanisms resulting in receptor-dependent transrepression have remained a mystery. However, recent pioneering work by Glass et al. has begun to elucidate the molecular mechanisms that control receptor-dependent transrepression of NF-κB responsive genes. These studies demonstrate that in the presence of PPAR-γ ligands, PPAR-γ can conjugate with small ubiquitin-like modifier-1 (SUMO1) resulting in the sumoylation of PPAR-γ. Sumoylated PPAR-γ binds the corepressor molecule NCoR which maintains the promoters of responsive genes in a repressed state, even in the presence of NF-κB activating stimuli. The mechanisms by which NF-κB responsive genes are believed to remain in a repressed state following sumoylation of PPAR-γ and consequent association with NCoR are believed to involve inhibition of the recruitment of ubiquitin conjugating enzymes to the corepressor complex following physical association of sumoylated PPAR-γ with NCoR [9–11]. Interestingly, recent studies have demonstrated that in addition to PPAR-γ, liver X receptor (LXR) mediated transrepression involves sumoylation of receptor and association of NCoR [12]. This suggests the possibility of a general mechanism of transrepression by PPARs and LXRs.

As stated above, PPAR-γ agonists can regulate gene expression in a receptor-dependent manner through receptor binding to PPREs or through receptor-dependent transrepression. In addition, PPAR-γ agonists including 15d-PGJ2 can regulate gene expression through receptor-independent mechanisms. For example, 15d-PGJ2 blocks I-κB degradation by inhibiting the activation of I-κB kinase resulting in the retention of NF-κB in the cytoplasm [13, 14]. In addition, 15d-PGJ_2 has been demonstrated to inhibit NF-κB binding to NF-κB DNA-response elements [15]. Thus, in summary, PPAR-γ agonists can regulate gene expression through both receptor-dependent and receptor-independent mechanisms.

2. EFFECTS OF PPAR-γ ON IMMUNE CELL FUNCTION

2.1. CNS resident cells

Microglia are bone marrow-derived cells that migrate to the CNS during embryonic development. Normally, these cells exist in a quiescent state in the CNS. Likewise, astrocytes, resident CNS cells that protect neurons through production of neurotrophic factors as well as uptake of glutamate and other neurotoxic molecules, commonly are quiescent in the CNS. However, these glial cells may become activated in response to insults including stress, trauma, and pathogens, and under these conditions may initiate protective immune responses. Upon activation, microglia and astrocytes produce proinflammatory molecules including nitric oxide (NO), cytokines, and chemokines. These proinflammatory molecules play critical roles in removing pathogens and debris from the infected or injured CNS. In contrast, chronically activated glia are believed to contribute to CNS damage characteristic of neuroinflammatory and neurodegenerative disorders including MS.

Van Eldik et al. were the first to evaluate the effects of PPAR-γ agonists on immune function in glial cells. They demonstrated that the PPAR-γ agonist 15d-PGJ_2 inhibited LPS induction of NO and iNOS expression in the murine BV-2 microglial cell line. However, troglitazone which is a PPAR-γ agonist and thiazolidinedione did not suppress LPS induction of these molecules. These results were interpreted to indicate that 15d-PGJ_2 functioned through a receptor-independent mechanism. Using the same BV-2 microglial cell system, the Van Eldik laboratory also demonstrated that 15d-PGJ_2 suppressed LPS induction of TNF-α, IL-1β, and COX-2 [16]. However, 15d-PGJ_2 increased intracellular glutathione levels as well as expression of heme oxygenase-1, an enzyme known to stimulate antioxidant production [17]. Minghetti et al. were the first to investigate the effects of PPAR-γ agonists on immune function in primary microglia. These studies indicated that 15d-PGJ_2 as well as the thiazolidinedione ciglitazone suppressed LPS induction of iNOS and TNF-α expression by primary rat microglia. These PPAR-γ agonists also suppressed IFN-γ induction of major histocompatibility class II in these cells. Because 15d-PGJ_2 and ciglitazone effects on microglial immune cell function were similar in these studies, it was interpreted that 15d-PGJ_2 functioned through a receptor-dependent mechanism [18].

More recently, we compared the effects of a series of thiazolidinediones or 15d-PGJ_2 on the production of proinflammatory molecules by primary microglia and astrocytes. These studies demonstrated that both thiazolidinediones and 15d-PGJ_2 inhibited the production of NO, the proinflammatory cytokines TNF-α, IL-1β, and IL-6, and the chemokine MCP-1 by these glial cells [19]. However,

even though 15d-PGJ_2 binds PPAR-γ with less affinity than each of the thiazolidinediones, this cyclopentenone prostaglandin more strongly inhibited production of these proinflammatory molecules by the glial cells, suggesting that 15d-PGJ_2 acts at least in part through receptor-independent mechanisms. A receptor-dependent effect of 15d-PGJ_2 in regulating glial cell immune function is supported by studies demonstrating that microglial cell activation is suppressed in a cooperative manner by this PPAR-γ ligand in combination with 9-cis retinoic acid, the ligand for the retinoic acid receptor RXR [20]. This supports the hypothesis that glial cell activation is maximally suppressed in the presence of ligands following formation of PPAR-γ/RXR heterodimers. Our observation that monocyte/microglia and astrocytes express increased levels of PPAR-γ during active EAE suggests that this receptor may modulate disease, perhaps through effects on glial cell activation [21]. Luna-Medina et al. [22] demonstrated that thiazolidinediones inhibited LPS induction of proinflammatory molecules by microglia and astrocytes. In addition, thiazolidinedione treatment of glial cultures suppressed the production of neurotoxic molecules. The effects of thiazolidinediones on glia in these studies were abrogated by PPAR-γ antagonists suggesting a receptor-dependent mechanism [22]. Minghetti et al. demonstrated that two flurbiprofen derivatives demonstrated to release NO suppressed glial cell activation through activation of PPAR-γ [23, 24]. Interestingly, one of these compounds, NXC 2216, initially activated the receptor, but later stimulated nitration and inactivation of PPAR-γ [24]. This suggested that these flurbiprofen derivatives could differentially activate or suppress glial immune cell function depending on length of treatment. Interestingly, PGA_2 potently suppressed microglia and astrocyte production of proinflammatory molecules [25]. Structurally, PGA_2 is a cyclopentenone prostaglandin like 15d-PGJ_2. However, PGA2 is not believed to bind PPAR-γ, suggesting that the cyclopentenone ring structure itself may modulate glial cell activation.

Astrocytes, like microglia, react to pathogens through a series of pattern recognition receptors which stimulate toll-like receptor signaling [26, 27]. Kielian et al. demonstrated that both 15d-PGJ_2 and the thiazolidinedione ciglitazone suppressed Staphylococcus aureus induction of NO and IL-1β by primary astrocytes. Suppression of these proinflammatory molecules by 15d-PGJ_2 and ciglitazone occurred in both wild-type and PPAR-γ deficient astrocytes, suggesting that these compounds mediated their effects in a receptor-independent manner [28].

PPAR-γ agonists have been demonstrated to modulate a variety of signaling pathways. Singh et al. showed that 15d-PGJ_2 inhibited LPS induction of NO and proinflammatory molecules in primary astrocytes. Transfection of wild-type PPAR-γ, dominant-negative PPAR-γ, or treatment of cells with a PPAR-γ antagonist did not alter 15d-PGJ_2 effects on astrocytes in these studies suggesting that the agonist functioned through a receptor-independent mechanism. The PPAR-γ agonist decreased NF-κB activity in these studies, presumably by inhibiting I-κB kinase. Singh et al. also demonstrated that 15d-PGJ_2 inhibited the phosphatidylinositol 3-kinase-Akt signaling pathway, suggesting an additional mechanism by which the agonist inhibited production of proinflammatory molecules in astrocytes [29]. Additional studies indicated that both the thiazolidinedione ciglitazone and 15d-PGJ_2 stimulated MAP kinase pathways in astrocytes through receptor-independent mechanisms involving production of reactive oxygen species [30]. The PPAR-γ agonists rosiglitazone and 15d-PGJ_2 also were demonstrated to modulate the JAK/STAT pathway by inhibiting the phosphorylation of specific JAK and STAT molecules following induced expression of suppressor of cytokine signaling (SOCS) 1 and 3 proteins in astrocytes and microglia [31].

Collectively, the studies discussed above suggest that PPAR-γ agonists may regulate immune function in glia through receptor-dependent or alternatively through receptor-independent mechanisms. Factors that may determine if receptor-dependent or receptor-independent mechanisms are employed may include the specific PPAR-γ agonist studied, the concentration of the agonist used, and the cell type studied. For example, responses are likely to differ between primary and transformed cells and may vary depending on the developmental state of the tissue from which the glial cells are derived.

The function and phenotype of T cells can be dramatically altered by glia. For example, the IL-12 family of cytokines which includes IL-12, IL-23, and IL-27 is believed to alter T cell phenotype and modulate the development of EAE and MS [32]. Specifically, the IL-12 family of cytokines modulates the differentiation of Th_1 cells which are believed to contribute to the development of EAE. In addition, IL-23 contributes to the production of Th_{17} cells which have recently been demonstrated to play a critical role in autoimmunity. IL-12 family members are heterodimeric. IL-12 consists of a dimer of p40 and p35 subunits. IL-23 consists of the same p40 subunit in association with p19. IL-27 exists as a dimer of p28 in association with EBV-induced molecule 3 (EBI3). Previously, we demonstrated that the PPAR-γ agonist 15d-PGJ_2 potently inhibited LPS induction of IL-12 p40 secretion by N9 mouse microglial cells and primary rat microglia [33]. More recently, we demonstrated that 15d-PGJ_2 and the thiazolidinedione rosiglitazone inhibited LPS induction of IL-12 p40, IL-12 p70, IL-23, and IL-27 p28 in primary microglia. In addition, 15d-PGJ_2 inhibited IL-12 p40, IL-23, and IL-27 p28, while rosiglitazone inhibited IL-23, and IL-27 p28, but not IL-12 p40 in primary astrocytes. LPS did not stimulate the production of IL-12 p70 in astrocytes [34]. These studies suggest that PPAR-γ agonists may modulate the development of EAE in part by modulating IL-12 family cytokine production by glia, which may alter T cell phenotype. Costimulatory molecules may be expressed by antigen presenting cells (APCs) including CNS microglia. Interaction of costimulatory molecules including CD40, CD80, and CD86 on APCs with their cognate receptors present of $CD4^+$ T cells is important in the activation and differentiation of these T cells, which likewise modulate the development of EAE and possibly MS. Our previous studies indicated that 15d-PGJ_2 inhibited microglial expression of CD40, but had no effect on the expression of CD80 and CD86 costimulatory molecules. Therefore, through modulation of costimulatory molecule expression

by microglia, PPAR-γ agonists may alter the pathogenesis of EAE [21, 35].

PPAR-γ agonists can alter the viability of neurons and oligodendrocytes, which are CNS cells compromised in MS. These agonists may alter the viability of these cells directly or indirectly by suppressing the production of cytotoxic molecules by activated microglia and astrocytes. Combs et al. [36] demonstrated that treatment of glial cultures with a variety of PPAR-γ agonists suppressed β-amyloid mediated toxicity of cortical neurons. Similarly, PPAR-γ agonists including thiazolidinediones and 15d-PGJ_2 inhibited LPS induction of neuronal cell death in studies utilizing a rat cortical neuron-glial coculture paradigm [37]. Furthermore, more recent studies indicated that thiazolidinediones treatment of cortical neuron-mixed glia cocultures resulted in protection of neurons. Neuron protection was abrogated in these studies by a PPAR-γ antagonist suggesting that neuron protection occurred by a receptor-dependent mechanism [22]. In addition to protecting neurons through suppression of glial activation, PPAR-γ agonists can also directly protect neurons. For example, PPAR-γ agonists have been demonstrated to protect neurons from a variety of neurotoxic agents including NMDA [38] and apolipoprotein E4 [39]. Interestingly, neurons express PPAR-γ and several studies suggest that neuron cell viability may be mediated through PPAR-γ activation [40, 41]. However, the mechanisms by which PPAR-γ regulates neuron cell viability have not been fully elucidated. Recent studies suggest that one mechanism by which PPAR-γ agonists may modulate neuron cell viability is through modulation of the antiapoptotic factor Bcl-2 [42]. Interestingly, PPAR-γ also regulates neural stem cell proliferation and differentiation [43]. Less is known concerning the role of PPAR-γ in modulating oligodendrocyte cell viability and differentiation. However, studies suggest that PPAR-γ protects oligodendrocyte progenitors [44] and modulates oligodendrocyte differentiation [45].

2.2. Peripheral immune cells

As mentioned previously, autoreactive T cells are believed to contribute to MS pathogenesis. Clark et al. initially demonstrated that T cells express PPAR-γ and that 15d-PGJ2 and ciglitazone inhibited T cell secretion of IL-2 and altered T cell proliferation [46]. PPAR-γ agonists have been demonstrated to induce apoptosis of T cells [47]. However, others studies indicate that PPAR-γ agonists promote the survival of T cells [48]. The exact reason for the discrepancy between these studies is not clear, but may involve differences in the concentration of PPAR-γ agonists used in the studies. Regulatory T cells play a critical role in suppressing the development of autoimmune diseases. Interestingly, recent studies indicate that PPAR-γ agonists enhance the generation and function of regulatory T cells [49, 50]. It is now clear that B cells have a significant role in modulating EAE and MS. Phipps et al. demonstrated that B cells express PPAR-γ and that PPAR-γ agonists stimulate the apoptosis of these cells [51, 52]. Collectively, these studies suggest that PPAR-γ may modulate MS in part by altering the viability and function of lymphocytes.

As stated previously, macrophages express PPAR-γ and PPAR-γ agonists regulate the function of these cells [53]. Like macrophages, dendritic cells are also of monocytic origin and function as professional antigen presenting cells. Dendritic cells also play a significant role in MS [54, 55]. Interestingly, PPAR-γ agonists have been shown to alter the viability and function of dendritic cells [56]. For example, cyclopentenone prostaglandins induced the apoptosis of dendritic cells, although apoptosis occurred through a receptor-independent mechanism [57]. PPAR-γ agonists were also shown to inhibit the migration of dendritic cells [58]. Furthermore, PPAR-γ agonists inhibited toll-like receptor mediated activation of dendritic cells by suppressing MAP kinase and NF-κB signaling pathways [59]. PPAR-γ effects on dendritic cell function have also been demonstrated to contribute to the development of $CD4^+$ T cell anergy [60]. Collectively, these studies indicate that PPAR-γ agonists may modulate MS in part through effects on monocytic cells.

Activated peripheral immune cells including antigen specific T cells and macrophages are capable of entering the CNS and contributing to MS pathology. Extravasation of peripheral immune cells is mediated by a variety of factors including chemokines and adhesion molecule expression on the cerebral vascular endothelium. Chemokines are synthesized under inflammatory conditions and generate a concentration gradient to which cells with the appropriate chemokine receptors migrate. The expression of specific chemokines is increased in EAE and MS [61, 62]. PPAR-γ agonists decrease the expression of MCP-1 which is a chemoattractant for monocytes and microglia [63, 64] as well as the T cell chemoattractants IP-10 (CXCL3), Mig (CXCL3), and I-TAC (CXCL3) [65]. Adhesion molecules present on the cerebral vascular endothelium facilitate extravasation of peripheral immune cells into the CNS. PPAR-γ agonists modulate the expression of various specific adhesion molecules suggesting an additional mechanism controlling immune cell extravasation into the CNS [66–68]. Future studies will be important in determining more detailed mechanisms by which PPAR-γ agonists modulate immune cell movement into the CNS.

3. EFFECTS OF PPAR-γ AGONISTS ON EAE AND MULTIPLE SCLEROSIS

3.1. EAE

EAE is a well-established animal model of MS. The disease is induced following immunization of CNS antigens, is mediated by myelin-specific T cells, and is characterized by CNS inflammation, demyelination, and remittent paralysis [1]. The blood-brain-barrier is believed compromised in EAE. However, the relative bioavailability of PPAR-γ agonists into the CNS varies, and it is important to consider this variable when interpreting studies designed to evaluate the effects on these agonists on EAE [69, 70]. The effects of PPAR-γ agonists in modulating EAE were first investigated by Niino et al. who demonstrated that the thiazolidinedione troglitazone inhibited the development of EAE elicited by

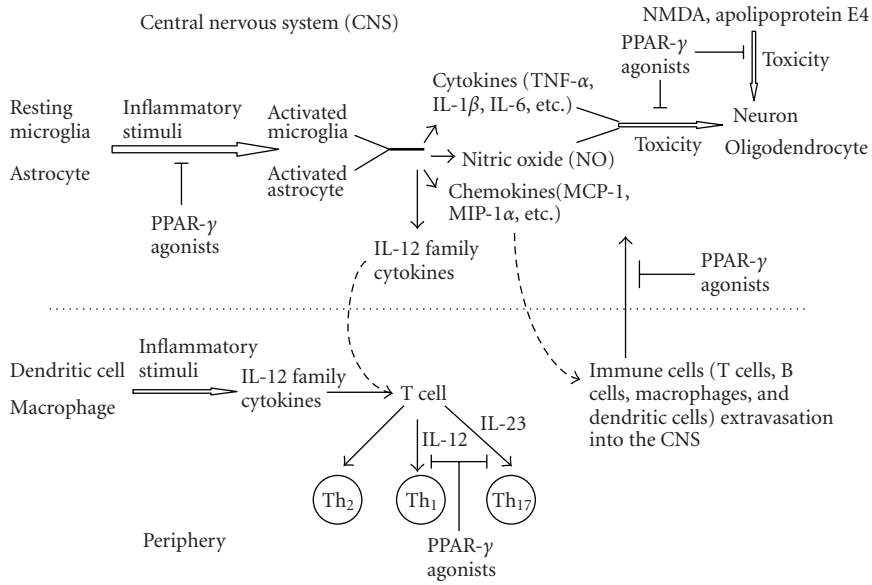

Figure 1: Effects of PPAR-γ on immune cell function. Glial cells including microglia and astrocytes may become activated in response to inflammatory stimuli including stress, trauma, and pathogens. Upon activation, microglia, and astrocytes produce a wide range of cytokines as well as NO. These molecules may be toxic to CNS cells, including myelin-producing oligodendrocytes and neurons, which are compromised in the course of MS. PPAR-γ agonists block the activation of glial cells resulting in repression of production of cytokines and NO, and protect oligodendrocytes and neurons from the toxic effects of these molecules. PPAR-γ agonists can also directly protect neurons from a variety of neurotoxic agents including NMDA and apolipoprotein E4. Chemokines secreted by activated glia cells establish a concentration gradient to which target cell populations migrate, and play important roles in recruiting cells into inflammatory sites in the CNS. PPAR-γ agonists may regulate the extravasation of peripheral immune cells into the CNS by suppressing chemokine expression. In addition, activated microglia serve as the major antigen-presenting cells (APCs) in the CNS, and dendritic cells and macrophages serve as APCs in the periphery. These APCs are capable of secreting IL-12 family cytokines upon activation. IL-12 and IL-23 play a critical role in the development of Th_1 and Th_{17} cells. PPAR-γ agonists may inhibit Th_1 and Th_{17} cell production by repressing IL-12 family cytokine secretion by these APCs, which is believed to protect against EAE/MS.

$MOG_{35–55}$ immunization of C57BL/6 mice. Troglitazone did not alter T cell proliferation or T cell production of IFN-γ in vitro in these studies [71]. We demonstrated that 15d-PGJ_2 inhibited the proliferation of splenic $MBP_{Ac1\text{-}11}$ transgenic T cells and inhibited IL-4 and IFN-γ production by these cells in vitro [21]. In vitro treatment of these transgenic T cells with 15d-PGJ_2 decreased the encephalitogenicity of these cells following adoptive transfer into naïve mice. This PPAR-γ agonist also inhibited the development of EAE when administered prior to or following onset of disease in an active model of disease involving immunization of B10.PL mice with $MBP_{Ac1\text{-}11}$ [21]. These studies suggest that PPAR-γ agonists may be effective in the treatment of established MS. Feinstein et al. demonstrated that monophasic EAE was inhibited by thiazolidinediones including pioglitazone. Although pioglitazone had no effect on the initial phase of relapsing-remitting EAE, disease severity was reduced upon subsequent relapses. In addition, these studies demonstrated that pioglitazone protected against axonal demyelination [72]. Bright et al. demonstrated that the PPAR-γ agonists 15d-PGJ_2 and ciglitazone decreased IL-12 expression and differentiation of Th_1 cells which was associated with decreased severity of active and passive EAE [73]. This team of investigators later showed that heterozygous PPAR-γ deficient mice demonstrated more severe EAE than wild-type mice [74]. They also demonstrated that more severe EAE developed following treatment with PPAR-γ antagonists [75]. We showed that a combination of the PPAR-γ agonist 15d-PGJ_2 and the RXR agonist 9-cis retinoic acid cooperatively inhibited the development of EAE [20]. Collectively, these studies support a role for PPAR-γ in modulating EAE. Homozygous PPAR-γ mutations are lethal thus complicating additional studies designed to evaluate the role of this receptor in modulating disease. Development of conditional PPAR-γ knockout mice as well as highly specific PPAR-γ antagonists will help define the role of PPAR-γ in modulation of EAE.

3.2. MS

Heneka et al. investigated the effects of thiazolidinediones pioglitazone and ciglitazone and the nonthiazolidinedione PPAR-γ agonist GW347845 on the function of peripheral blood mononuclear cells (PBMCs) from MS patients and healthy donors. These studies demonstrated that all of these PPAR-γ agonists decreased phytohemagglutinin (PHA) induced T cell proliferation and production of the cytokines TNF-α and IFN-γ by PBMCs. Interestingly, proliferation and cytokine secretion were further suppressed following pretreatment of cells with PPAR-γ agonists. These studies

also demonstrated that the PPAR-γ agonists decreased bcl-2 expression and induced apoptosis of activated T cells [76]. Additional studies by the same group indicated that PPAR-γ expression was reduced in PBMCs from MS patients relative to healthy donors, which correlated with decreased anti-inflammatory effects of pioglitazone on patient-derived PBMCs [77]. PHA stimulation of PBMCs from healthy donors resulted in decreased PPAR-γ expression, which was overcame by pretreatment of these cells with PPAR-γ agonist. Long-term treatment of diabetes patients with pioglitazone also overcame the decrease in PPAR-γ expression in PHA treated PBMCs from these patients. These studies indicate that pioglitazone treatment in humans can protect against loss of PPAR-γ expression resulting from inflammation. These studies also indicated that preincubation of PBMCs with pioglitazone resulted in increased PPAR-γ and decreased NF-κB DNA-binding activity [77]. Collectively, these studies suggest that sustained activation of PPAR-γ may prevent inflammation induced reduction of the expression of this receptor. These studies may have important implications concerning the use of PPAR-γ agonists in the treatment of MS.

A small clinical study supports the idea that PPAR-γ agonists may be effective in the treatment of MS [78]. Larger scale clinical trials are currently underway to further assess the therapeutic potential of PPAR-γ agonists for the treatment of MS.

4. CONCLUSIONS

PPAR-γ agonists have been demonstrated to limit pathology in animal models of human neuroinflammatory and neurodegenerative disorders. These studies suggest that these agonists may be effective in the treatment of human diseases including MS. Type II diabetes is commonly treated with PPAR-γ agonists termed thiazolidinediones which include pioglitazone and rosiglitazone. These medications have an excellent safety profile which should facilitate future clinical trials designed to evaluate the efficacy of these PPAR-γ agonists in the treatment of human disorders of the CNS. However, basic research designed to better understand the cellular and molecular mechanisms by which PPAR-γ agonists regulate CNS inflammation will be critical in developing more effective treatment strategies for neuroinflammatory disorders including MS.

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REFERENCES

[1] R. Martin, H. F. McFarland, and D. E. McFarlin, "Immunological aspects of demyelinating diseases," *Annual Review of Immunology*, vol. 10, pp. 153–187, 1992.

[2] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[3] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[4] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[5] D. S. Straus and C. K. Glass, "Cyclopentenone prostaglandins: new insights on biological activities and cellular targets," *Medicinal Research Reviews*, vol. 21, no. 3, pp. 185–210, 2001.

[6] Y. Kamei, L. Xu, T. Heinzel, et al., "A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors," *Cell*, vol. 85, no. 3, pp. 403–414, 1996.

[7] T. K. Kerppola, D. Luk, and T. Curran, "Fos is a preferential target of glucocorticoid receptor inhibition of AP-1 activity in vitro," *Molecular and Cellular Biology*, vol. 13, no. 6, pp. 3782–3791, 1993.

[8] M. Li, G. Pascual, and C. K. Glass, "Peroxisome proliferator-activated receptor γ-dependent repression of the inducible nitric oxide synthase gene," *Molecular and Cellular Biology*, vol. 20, no. 13, pp. 4699–4707, 2000.

[9] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[10] M. Ricote and C. K. Glass, "PPARs and molecular mechanisms of transrepression," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 926–935, 2007.

[11] D. S. Straus and C. K. Glass, "Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms," *Trends in Immunology*, vol. 28, no. 12, pp. 551–558, 2007.

[12] S. Ghisletti, W. Huang, S. Ogawa, et al., "Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARγ," *Molecular Cell*, vol. 25, no. 1, pp. 57–70, 2007.

[13] A. Castrillo, M. J. M. Díaz-Guerra, S. Hortelano, P. Martín-Sanz, and L. Boscá, "Inhibition of IκB kinase and IκB phosphorylation by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in activated murine macrophages," *Molecular and Cellular Biology*, vol. 20, no. 5, pp. 1692–1698, 2000.

[14] A. Rossi, P. Kapahi, G. Natoli, et al., "Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase," *Nature*, vol. 403, no. 6765, pp. 103–108, 2000.

[15] D. S. Straus, G. Pascual, M. Li, et al., "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 9, pp. 4844–4849, 2000.

[16] T. V. Petrova, K. T. Akama, and L. J. Van Eldik, "Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 8, pp. 4668–4673, 1999.

[17] T. Koppal, T. V. Petrova, and L. J. Van Eldik, "Cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 acts as a general inhibitor of inflammatory responses in activated BV-2 microglial cells," *Brain Research*, vol. 867, no. 1-2, pp. 115–121, 2000.

[18] A. Bernardo, G. Levi, and L. Minghetti, "Role of the peroxisome proliferator-activated receptor-γ (PPAR-γ) and its natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in the regulation of microglial functions," *European Journal of Neuroscience*, vol. 12, no. 7, pp. 2215–2223, 2000.

[19] P. D. Storer, J. Xu, J. Chavis, and P. D. Drew, "Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroimmunology*, vol. 161, no. 1-2, pp. 113–122, 2005.

[20] A. Diab, R. Z. Hussain, A. E. Lovett-Racke, J. A. Chavis, P. D. Drew, and M. K. Racke, "Ligands for the peroxisome proliferator-activated receptor-γ and the retinoid X receptor exert additive anti-inflammatory effects on experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 148, no. 1-2, pp. 116–126, 2004.

[21] A. Diab, C. Deng, J. D. Smith, et al., "Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis," *Journal of Immunology*, vol. 168, no. 5, pp. 2508–2515, 2002.

[22] R. Luna-Medina, M. Cortes-Canteli, M. Alonso, A. Santos, A. Martínez, and A. Perez-Castillo, "Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor γ activation," *Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21453–21462, 2005.

[23] A. Bernardo, M. A. Ajmone-Cat, L. Gasparini, E. Ongini, and L. Minghetti, "Nuclear receptor peroxisome proliferator-activated receptor-γ is activated in rat microglial cells by the anti-inflammatory drug HCT1026, a derivative of flurbiprofen," *Journal of Neurochemistry*, vol. 92, no. 4, pp. 895–903, 2005.

[24] A. Bernardo, L. Gasparini, E. Ongini, and L. Minghetti, "Dynamic regulation of microglial functions by the non-steroidal anti-inflammatory drug NCX 2216: implications for chronic treatments of neurodegenerative diseases," *Neurobiology of Disease*, vol. 22, no. 1, pp. 25–32, 2006.

[25] P. D. Storer, J. Xu, J. A. Chavis, and P. D. Drew, "Cyclopentenone prostaglandins PGA_2 and 15-deoxy-$\delta^{12,14}PGJ_2$ suppress activation of marine microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroscience Research*, vol. 80, no. 1, pp. 66–74, 2005.

[26] T. Kielian, "Toll-like receptors in central nervous system glial inflammation and homeostasis," *Journal of Neuroscience Research*, vol. 83, no. 5, pp. 711–730, 2006.

[27] C. Farina, F. Aloisi, and E. Meinl, "Astrocytes are active players in cerebral innate immunity," *Trends in Immunology*, vol. 28, no. 3, pp. 138–145, 2007.

[28] N. K. Phulwani, D. L. Feinstein, V. Gavrilyuk, C. Akar, and T. Kielian, "15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2) and ciglitazone modulate *Staphylococcus aureus*-dependent astrocyte activation primarily through a PPAR-γ-independent pathway," *Journal of Neurochemistry*, vol. 99, no. 5, pp. 1389–1402, 2006.

[29] S. Giri, R. Rattan, A. K. Singh, and I. Singh, "The 15-deoxy-$\delta^{12,14}$-prostaglandin J_2 inhibits the inflammatory response in primary rat astrocytes via down-regulating multiple steps in phosphatidylinositol 3-kinase-akt-NF-κB-p300 pathway independent of peroxisome proliferator-activated receptor γ," *Journal of Immunology*, vol. 173, no. 8, pp. 5196–5208, 2004.

[30] A. M. Lennon, M. Ramaugé, A. Dessouroux, and M. Pierre, "MAP kinase cascades are activated in astrocytes and preadipocytes by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and the thiazolidinedione ciglitazone through peroxisome proliferator activator receptor γ-independent mechanisms involving reactive oxygenated species," *Journal of Biological Chemistry*, vol. 277, no. 33, pp. 29681–29685, 2002.

[31] E. J. Park, S. Y. Park, E.-H. Joe, and I. Jou, "15d-PGJ_2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia," *Journal of Biological Chemistry*, vol. 278, no. 17, pp. 14747–14752, 2003.

[32] B. Gran, G.-X. Zhang, and A. Rostami, "Role of the IL-12/IL-23 system in the regulation of T-cell responses in central nervous system inflammatory demyelination," *Critical Reviews in Immunology*, vol. 24, no. 2, pp. 111–128, 2004.

[33] P. D. Drew and J. A. Chavis, "The cyclopentone prostaglandin 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 represses nitric oxide, TNF-α, and IL-12 production by microglial cells," *Journal of Neuroimmunology*, vol. 115, no. 1-2, pp. 28–35, 2001.

[34] J. Xu and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia," *Journal of Immunology*, vol. 178, no. 3, pp. 1904–1913, 2007.

[35] T. Kielian, M. McMahon, E. D. Bearden, A. C. Baldwin, P. D. Drew, and N. Esen, "*S. aureus*-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2)," *Journal of Neurochemistry*, vol. 90, no. 5, pp. 1163–1172, 2004.

[36] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of β-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists," *Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[37] E. J. Kim, K. J. Kwon, J.-Y. Park, S. H. Lee, C.-H. Moon, and E. J. Baik, "Effects of peroxisome proliferator-activated receptor agonists on LPS-induced neuronal death in mixed cortical neurons: associated with iNOS and COX-2," *Brain Research*, vol. 941, no. 1-2, pp. 1–10, 2002.

[38] X. Zhao, Z. Ou, J. C. Grotta, N. Waxham, and J. Aronowski, "Peroxisome-proliferator-activated receptor-gamma (PPARγ) activation protects neurons from NMDA excitotoxicity," *Brain Research*, vol. 1073-1074, no. 1, pp. 460–469, 2006.

[39] J. Brodbeck, M. E. Balestra, A. M. Saunders, A. D. Roses, R. W. Mahley, and Y. Huang, "Rosiglitazone increases dendritic spine density and rescues spine loss caused by apolipoprotein E4 in primary cortical neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 4, pp. 1343–1346, 2008.

[40] S. A. Smith, G. R. Monteith, N. A. Holman, J. A. Robinson, F. J. May, and S. J. Roberts-Thomson, "Effects of peroxisome proliferator-activated receptor γ ligands ciglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on rat cultured cerebellar granule neuronal viability," *Journal of Neuroscience Research*, vol. 72, no. 6, pp. 747–755, 2003.

[41] N. C. Inestrosa, J. A. Godoy, R. A. Quintanilla, C. S. Koenig, and M. Bronfman, "Peroxisome proliferator-activated receptor γ is expressed in hippocampal neurons and its activation prevents β-amyloid neurodegeneration: role of Wnt signaling," *Experimental Cell Research*, vol. 304, no. 1, pp. 91–104, 2005.

[42] K. Fuenzalida, R. Quintanilla, P. Ramos, et al., "Peroxisome proliferator-activated receptor γ up-regulates the Bcl-2 anti-apoptotic protein in neurons and induces mitochondrial stabilization and protection against oxidative stress and apoptosis," *Journal of Biological Chemistry*, vol. 282, no. 51, pp. 37006–37015, 2007.

[43] K. Wada, A. Nakajima, K. Katayama, et al., "Peroxisome proliferator-activated receptor γ-mediated regulation of neural stem cell proliferation and differentiation," *Journal of Biological Chemistry*, vol. 281, no. 18, pp. 12673–12681, 2006.

[44] A. S. Paintlia, M. K. Paintlia, I. Singh, and A. K. Singh, "IL-4-induced peroxisome proliferator-activated receptor γ activation inhibits NF-κB trans activation in central nervous system (CNS) glial cells and protects oligodendrocyte progenitors under neuroinflammatory disease conditions: implication for CNS-demyelinating diseases," *Journal of Immunology*, vol. 176, no. 7, pp. 4385–4398, 2006.

[45] A. D. Roth, A. V. Leisewitz, J. E. Jung, et al., "PPAR γ activators induce growth arrest and process extension in B12 oligodendrocyte-like cells and terminal differentiation of cultured oligodendrocytes," *Journal of Neuroscience Research*, vol. 72, no. 4, pp. 425–435, 2003.

[46] R. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[47] S. G. Harris and R. P. Phipps, "The nuclear receptor PPAR gamma is expressed by mouse T lymphocytes and PPAR gamma agonists induce apoptosis," *European Journal of Immunology*, vol. 31, no. 4, pp. 1098–1105, 2001.

[48] S.-H. Jo, C. Yang, Q. Miao, et al., "Peroxisome proliferator-activated receptor γ promotes lymphocyte survival through its actions on cellular metabolic activities," *Journal of Immunology*, vol. 177, no. 6, pp. 3737–3745, 2006.

[49] R. Hontecillas and J. Bassaganya-Riera, "Peroxisome proliferator-activated receptor γ is required for regulatory $CD4^+$ T cell-mediated protection against colitis," *Journal of Immunology*, vol. 178, no. 5, pp. 2940–2949, 2007.

[50] E. A. Wohlfert, F. C. Nichols, E. Nevius, and R. B. Clark, "Peroxisome proliferator-activated receptor γ (PPARγ) and immunoregulation: enhancement of regulatory T cells through PPARγ- dependent and -independent mechanisms," *Journal of Immunology*, vol. 178, no. 7, pp. 4129–4135, 2007.

[51] J. Padilla, K. Kaur, H. J. Cao, T. J. Smith, and R. P. Phipps, "Peroxisome proliferator activator receptor-γ agonists and 15-deoxy-$\Delta^{12,14}$-PGJ_2 induce apoptosis in normal and malignant B-lineage cells," *Journal of Immunology*, vol. 165, no. 12, pp. 6941–6948, 2000.

[52] J. Padilla, E. Leung, and R. P. Phipps, "Human B lymphocytes and B lymphomas express PPAR-γ and are killed by PPAR-γ agonists," *Clinical Immunology*, vol. 103, no. 1, pp. 22–33, 2002.

[53] A. Castrillo and P. Tontonoz, "Nuclear receptors in macrophage biology: at the crossroads of lipid metabolism and inflammation," *Annual Review of Cell and Developmental Biology*, vol. 20, pp. 455–480, 2004.

[54] S. L. Manuel, S. Rahman, B. Wigdahl, Z. K. Khan, and P. Jain, "Dendritic cells in autoimmune diseases and neuroinflammatory disorders," *Frontiers in Bioscience*, vol. 12, pp. 4315–4335, 2007.

[55] G. F. Wu and T. M. Laufer, "The role of dendritic cells in multiple sclerosis," *Current Neurology and Neuroscience Reports*, vol. 7, no. 3, pp. 245–252, 2007.

[56] I. Szatmari, E. Rajnavolgyi, and L. Nagy, "PPARγ, a lipid-activated transcription factor as a regulator of dendritic cell function," *Annals of the New York Academy of Sciences*, vol. 1088, pp. 207–218, 2006.

[57] A. Nencioni, K. Lauber, F. Grünebach, et al., "Cyclopentenone prostaglandins induce caspase activation and apoptosis in dendritic cells by a PPAR-γ-independent mechanism: regulation by inflammatory and T cell-derived stimuli," *Experimental Hematology*, vol. 30, no. 9, pp. 1020–1028, 2002.

[58] V. Angeli, H. Hammad, B. Staels, M. Capron, B. N. Lambrecht, and F. Trottein, "Peroxisome proliferator-activated receptor γ inhibits the migration of dendritic cells: consequences for the immune response," *Journal of Immunology*, vol. 170, no. 10, pp. 5295–5301, 2003.

[59] S. Appel, V. Mirakaj, A. Bringmann, M. M. Weck, F. Grünebach, and P. Brossart, "PPAR-γ agonists inhibit toll-like receptor-mediated activation of dendritic cells via the MAP kinase and NF-κB pathways," *Blood*, vol. 106, no. 12, pp. 3888–3894, 2005.

[60] L. Klotz, I. Dani, F. Edenhofer, et al., "Peroxisome proliferator-activated receptor γ control of dendritic cell function contributes to development of $CD4^+$ T cell anergy," *Journal of Immunology*, vol. 178, no. 4, pp. 2122–2131, 2007.

[61] N. Godessart and S. L. Kunkel, "Chemokines in autoimmune disease," *Current Opinion in Immunology*, vol. 13, no. 6, pp. 670–675, 2001.

[62] C. Trebst and R. M. Ransohoff, "Investigating chemokines and chemokine receptors in patients with multiple sclerosis: opportunities and challenges," *Archives of Neurology*, vol. 58, no. 12, pp. 1975–1980, 2001.

[63] U. Kintscher, S. Goetze, S. Wakino, et al., "Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes," *European Journal of Pharmacology*, vol. 401, no. 3, pp. 259–270, 2000.

[64] X. Zhang, J. M. Wang, W. H. Gong, N. Mukaida, and H. A. Young, "Differential regulation of chemokine gene expression by 15-deoxy-$\Delta^{12,14}$ prostaglandin $J_2^{1,2}$," *Journal of Immunology*, vol. 166, no. 12, pp. 7104–7111, 2001.

[65] N. Marx, F. Mach, A. Sauty, et al., "Peroxisome proliferator-activated receptor-γ activators inhibit IFN-γ- induced expression of the T cell-active CXC chemokines IP-10, Mig, and I-TAC in human endothelial cells," *Journal of Immunology*, vol. 164, no. 12, pp. 6503–6508, 2000.

[66] N.-G. Chen, S. F. Sarabia, P. J. Malloy, X.-Y. Zhao, D. Feldman, and G. M. Reaven, "PPARγ agonists enhance human vascular endothelial adhesiveness by increasing ICAM-1 expression," *Biochemical and Biophysical Research Communications*, vol. 263, no. 3, pp. 718–722, 1999.

[67] S. M. Jackson, F. Parhami, X.-P. Xi, et al., "Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 19, no. 9, pp. 2094–2104, 1999.

[68] N.-G. Chen and X. Han, "Dual function of troglitazone in ICAM-1 gene expression in human vascular endothelium," *Biochemical and Biophysical Research Communications*, vol. 282, no. 3, pp. 717–722, 2001.

[69] Y. Maeshiba, Y. Kiyota, K. Yamashita, Y. Yoshimura, M. Motohashi, and S. Tanayama, "Disposition of the new antidiabetic agent pioglitazone in rats, dogs, and monkeys," *Arzneimittel-Forschung*, vol. 47, no. 1, pp. 29–35, 1997.

[70] M. E. Risner, A. M. Saunders, J. F. B. Altman, et al., "Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease," *Pharmacogenomics Journal*, vol. 6, no. 4, pp. 246–254, 2006.

[71] M. Niino, K. Iwabuchi, S. Kikuchi, et al., "Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-γ," *Journal of Neuroimmunology*, vol. 116, no. 1, pp. 40–48, 2001.

[72] D. L. Feinstein, E. Galea, V. Gavrilyuk, et al., "Peroxisome proliferator-activated receptor-γ agonists prevent experimental autoimmune encephalomyelitis," *Annals of Neurology*, vol. 51, no. 6, pp. 694–702, 2002.

[73] C. Natarajan and J. J. Bright, "Peroxisome proliferator-activated receptor-gamma agonist inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation," *Genes & Immunity*, vol. 3, no. 2, pp. 59–70, 2002.

[74] C. Natarajan, G. Muthian, Y. Barak, R. M. Evans, and J. J. Bright, "Peroxisome proliferator-activated receptor-γ-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis," *Journal of Immunology*, vol. 171, no. 11, pp. 5743–5750, 2003, Erratum in *Journal of Immunology*, vol. 172, no. 8, p. 5127, 2004.

[75] H. P. Raikwar, G. Muthian, J. Rajasingh, C. Johnson, and J. J. Bright, "PPARγ antagonists exacerbate neural antigen-specific Th1 response and experimental allergic encephalomyelitis," *Journal of Neuroimmunology*, vol. 167, no. 1-2, pp. 99–107, 2005.

[76] S. Schmidt, E. Moric, M. Schmidt, M. Sastre, D. L. Feinstein, and M. T. Heneka, "Anti-inflammatory and antiproliferative actions of PPAR-γ agonists on T lymphocytes derived from MS patients," *Journal of Leukocyte Biology*, vol. 75, no. 3, pp. 478–485, 2004.

[77] L. Klotz, M. Schmidt, T. Giese, et al., "Proinflammatory stimulation and pioglitazone treatment regulate peroxisome proliferator-activated receptor γ levels in peripheral blood mononuclear cells from healthy controls and multiple sclerosis patients," *Journal of Immunology*, vol. 175, no. 8, pp. 4948–4955, 2005.

[78] H. A. Pershadsingh, M. T. Heneka, R. Saini, N. M. Amin, D. J. Broeske, and D. L. Feinstein, "Effect of pioglitazone treatment in a patient with secondary multiple sclerosis," *Journal of Neuroinflammation*, vol. 1, article 3, pp. 1–4, 2004.

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Review Article

PPARs in Alzheimer's Disease

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Peroxisome proliferator-activated receptors (PPARs) are well studied for their peripheral physiological and pathological impact, but they also play an important role for the pathogenesis of various disorders of the central nervous system (CNS) like multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's, and Parkinson's disease. The observation that PPARs are able to suppress the inflammatory response in peripheral macrophages and in several models of human autoimmune diseases lead to the idea that PPARs might be beneficial for CNS disorders possessing an inflammatory component. The neuroinflammatory response during the course of Alzheimer's disease (AD) is triggered by the neurodegeneration and the deposition of the β-amyloid peptide in extracellular plaques. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been considered to delay the onset and reduce the risk to develop Alzheimer's disease, while they also directly activate PPARγ. This led to the hypothesis that NSAID protection in AD may be partly mediated by PPARγ. Several lines of evidence have supported this hypothesis, using AD-related transgenic cellular and animal models. Stimulation of PPARγ receptors by synthetic agonist (thiazolidinediones) inducing anti-inflammatory, anti-amyloidogenic, and insulin sensitising effects may account for the observed effects. Several clinical trials already revealed promising results using PPAR agonists, therefore PPARs represent an attractive therapeutic target for the treatment of AD.

1. INTRODUCTION

The peroxisome proliferator-activated receptors (PPARs) belong to the family of nuclear hormone receptors (NHR) that comprise 48 human ligand-inducible transcription factors, which activity is regulated by steroids and lipid metabolites. Three different PPAR genes (PPARα, PPARβ, also called δ, and PPARγ) have been identified in all metazoa, that show unique spatiotemporal tissue-dependent patterns of expression during fetal development in a variety of cell types deriving form the ecto-, meso- or endoderm in rodents. Functionally PPARs are involved in adipocyte differentiation, lipid storage, and glucose homeostasis of the adipose tissue, brain, placenta, and skin (reviewed in [1]).

1.1. Functions of PPARs

PPARs act principally as lipid sensors and regulate the whole body metabolism in response to dietary lipid intake and direct their subsequent metabolism and storage [2]. The prototypic member of the family, PPARα, was initially reported to be induced by peroxisome proliferators, and now denotes the subfamily of three related receptors. The natural ligands of these receptors are dietary lipids and their metabolites. The specific ligands have been difficult to establish, owing to the relatively low affinity interactions and broad ligand specificity of the receptors.

PPARα acts primarily to regulate energy homoeostasis through its ability to stimulate the breakdown of fatty acids and cholesterol, driving gluconeogenesis and reduction in serum triglyceride levels. This receptor acts as a lipid sensor, binding fatty acids and initiating their subsequent metabolism. PPARγ binds a number of lipids including fatty acids, eicosanoids, and other natural lipid ligands. Its dominant action is to stimulate adipocyte differentiation and to direct lipid metabolites to be deposited in this tissue. PPARγ operates at the critical metabolic intersection of lipid and carbohydrate metabolism. PPARγ activation is linked to reduction in serum glucose levels, likely as a secondary effect of its ability to regulate endocrine factors. It is this latter activity that has led to the development of specific PPARγ agonists for the treatment of type II diabetes [3]. The

PPARβ/δ binds and responds to VLDL-derived fatty acids, eicosanoids including prostaglandin A1 [4] and appears to be primarily involved in fatty acid oxidation, particularly in muscle.

PPARs regulate gene expression by forming heterodimers with retinoid-X-receptors (RXRs). Stimulation of target gene expression is controlled by specific PPAR-response elements in the promoter region (PPREs). Under unstimulated conditions, these heterodimers are associated with corepressors, like N-CoR and SMRT, which suppress gene transcription [1]. Upon ligand binding to the nuclear receptor, the corepressors are displaced and transcriptional coactivators are recruited to the receptor. These coactivator receptor complexes finally induce the formation of a much larger transcriptional complex which subsequently links the basal transcriptional apparatus and initiates gene transcription. In addition, activity of PPARs is also regulated by post-translational modification such as phosphorylation and sumoylation [5, 6].

Like other NHR, PPARs also inhibit proinflammatory gene expression by a controversial mechanism of *transcriptional transrepression,* which is not mediated by their binding to PPREs. PPARγ is able to suppress expression of proinflammatory genes in myeloid lineage cells, such as microglia and macrophages, and in the vasculature [7], by suppressing the action of NFκB, AP-1, and STAT1 transcription factors [8]. A mechanistic model for the PPARγ-mediated transrepression has recently been proposed. NFκB-regulated inflammatory genes are maintained under basal conditions in a repressed state by N-Cor containing corepressor complexes. Upon exposure to proinflammatory stimuli this complex is dismissed and gene expression is initiated. This dismissal can be prevented by sumoylated PPARγ: PPARγ agonist complexes that stabilizes NCor complexes at the promoters of NFκB-regulated genes, thus preventing inflammatory gene expression [9, 10].

Binding of PPARs to their specific ligands leads to conformational changes which allow corepressor release and coactivator recruitment. Even though all PPARs can be attributed to a common ancestral nuclear receptor, each PPAR isotype has its own properties with regard to ligand binding. Synthetic thiazolidinediones (TZDs), which are commonly prescribed for the treatment of type II diabetes, are selective PPARγ ligands. Naturally occurring PPARγ ligands include eicosanoids and the prostaglandin 15d-PGJ2. The best characterized PPARγ agonists are the TZDs including pioglitazone and rosiglitazone which are Food and Drug Administration (FDA) approved for treatment of type II diabetes and troglitazone, which was withdrawn in 2000. PPARα ligands include fibrates that are commonly used for the treatment of hypertriglyceridemia and the synthetic agonists WY14,643, and GW7647. PPARβ/δ agonists include the prostacyclin PGI2, and synthetic agents including GW0742, GW501516, and GW7842. All three PPAR isotypes can be activated by polyunsaturated fatty acids with different affinities and efficiencies [11]. An overview addressing the affinity of several natural and synthetic ligands has recently been summarized [12].

1.2. PPARs during development

PPARα and γ transcripts appear late during fetal development of rat and mouse (day 13.5 of gestation), with similar expression pattern to their adult distribution. PPARα is found in the liver, the kidney, the intestine, the heart, the skeletal muscle, the adrenal gland, and the pancreas. PPARγ expression is restricted to the brown adipose tissue (day 18.5 of gestation), and to the CNS (day 13.5 to 15.5 of gestation). Compared to the two other isotypes, PPARβ/δ is expressed ubiquitously and earlier during fetal development [13]. In adult rodent organs, the distribution of PPARα is similar to its fetal pattern of expression.

Not much is known about the expression of the PPARs during human development [14–16]. PPARα is most highly expressed in tissues that catabolise fatty acids, such as the adult liver, heart, kidney, large intestine, and skeletal muscle. PPARβ/δ mRNA is present ubiquitously, with a higher expression in the digestive tract and the placenta. PPARγ is abundantly expressed in the white adipose tissue, and is present at lower levels in the skeletal muscle, the heart, and the liver. Surprisingly, and in contrast to rodents, human PPARγ seems to be absent from lymphoid tissues, even though PPARγ has been shown to be present in macrophages in human atheroma.

1.3. PPARs in the brain

All three PPAR isotypes are coexpressed in the nervous system during late rat embryogenesis, and PPARβ/δ is the prevalent isotype. The expression of the three PPAR isotypes peaks in the rat CNS between day 13.5. and 18.5 of gestation. Whereas PPARβ/δ remains highly expressed in this tissue, the expression of PPARα and γ decreases postnatally in the brain [17]. While PPARβ/δ has been found in neurons of numerous brain areas, PPARα and γ have been localized to more restricted brain areas [18, 19]. Analysis of the expression of PPARs in different brain regions of adult mice revealed that PPARβ/δ mRNAs are preferentially found in the cerebellum, the brain stem, and the cortex, whereas PPARγ mRNAs are enriched in the olfactory areas as well as in the cortex. Expression of all three isotypes was found to be low to moderate in the hippocampus. More detailed analysis of PPARs expression within the hippocampus by in situ hybridisation revealed a ubiquitous expression pattern for PPARα, whereas PPARβ was found to be enriched in the dentate gyrus/CA1 region and PPARγ expression was restricted to the CA3 region [20].

Even though this pattern of expression, which is isotype specific and regulated during development, suggests that the PPARs may play a role during the formation of the CNS, their function in this tissue are still poorly understood. Both in vitro and in vivo observations show that PPARβ/δ is the prevalent isoform in the brain, and is found in all cell types, whereas PPARα is expressed at very low levels predominantly in astrocytes [21]. Acyl-CoA synthetase 2, which is crucial in fatty acid utilization, is regulated by PPARβ/δ at the transcriptional level, providing a facile measure of PPARβ/δ action. This observation strongly suggests that PPARβ/δ

participates in the regulation of lipid metabolism in the brain. This hypothesis is further supported by the observation that PPARβ/δ null mice exhibit an altered myelination of the corpus callosum. Such a defect was not observed in other regions of the central nervous system, and the expression of mRNA encoding proteins involved in the myelination process remained unchanged in the brain.

Expression of all PPAR isoforms, including PPARγ, has been confirmed in the adult brain. Furthermore, it has been suggested that PPAR activation in neurons may directly influence neuron cell viability and differentiation [22–26]. The localization of PPARs has also been investigated in purified cultures of neural cells. PPARβ/δ is expressed in immature oligodendrocytes and its activation promotes differentiation, myelin maturation, and turnover [27, 28]. The PPARγ is the dominant isoform in microglia. Astrocytes possess all three PPAR isotypes, although to different degrees depending on the brain area and animal age [29, 30]. The role of PPARs in the CNS is mainly been related to lipid metabolism, however, these receptors, especially PPARγ, have been implicated in neural cell differentiation and death as well as in inflammation and neurodegeneration [23]. PPARα has been suggested to be involved in the acetylcholine metabolism [31] and to be related to excitatory amino acid neurotransmission and oxidative stress defence [18].

2. INFLAMMATION AND ALZHEIMER'S DISEASE

The number of individuals with the Alzheimer's disease (AD) is dramatically increasing throughout the developed world. The large number of affected individuals and the increasing prevalence of the disease presents a substantial challenge to health care systems and does so in the face of substantial economic costs. The pathological hallmarks of AD are the formation of extracellular plaques consisting of amyloid-β peptides and intracellular neurofibrillary tangles made up from hyperphosphorylated tau protein, causing neuronal death that is responsible for progressive memory loss and inexorable decline of cognitive functions [32, 33]. Analysis of the genetic forms and animal models suggested a pivotal role for the amyloid β peptide (Aβ), nevertheless, the biological basis of AD, especially of the sporadic forms, is still poorly understood. Genetically, Aβ metabolism is closely linked to lipid metabolism as a certain allele of the lipid carrier protein ApoE is associated with significantly increased risk for AD [34]. Another key hallmark of AD brain is the presence of chronic neuroinflammation without any signs of leukocyte infiltration. Amyloid plaques within the brain are populated by abundant, activated microglia, and astrocytes [35]. Microglial activation is accompanied by the secretion of inflammatory cytokines and chemokines including interleukin (IL)-1β, IL-6, monocyte chemotactic protein-1, (MCP-1), and tumor necrosis factor (TNF)-α [36]. It was posited that activation of microglia and the concurrent production of inflammatory molecules may deteriorate and accelerate the progression of AD and therefore the neuronal loss [35]. Neuronal expression of inflammatory enzyme systems, including iNOS, has also been described in AD [37–39]. Altogether, these data suggest that anti-inflammatory therapies may be beneficial for AD treatment (see Figure 1).

3. EFFECTS OF PPARγ AGONISTS ON ALZHEIMER'S DISEASE

PPARγ is expressed in the brain at the low levels under physiological conditions. Recently, a detailed gene expression analysis has demonstrated that mRNA levels are elevated in AD patients [40]. This suggests that PPARγ plays a role in the modulation of the pathophysiology of AD. Currently used drugs are mainly targeted at symptomatic improvement of the patients. These agents have only modest therapeutic efficacy over rather short periods. Thus, the development of new therapeutic approaches is of critical importance.

The initial studies exploring the actions of PPARγ in AD were based on the ability of nonsteroidal anti-inflammatory drugs (NSAID) to activate this receptor. A number of epidemiological studies demonstrated that NSAID treatment reduces AD risk by as much as 80% and it was suggested that these effects arise from the ability of these drugs to stimulate PPARγ and to inhibit inflammatory responses in the AD brain [41–45]. This hypothesis is supported by the finding that experimental expression of iNOS in neurons resulted in time-dependent neuronal cell death which was prevented by activation of PPARγ in vitro and in vivo [23, 46]. In addition, PPARγ activation in microglial cells suppressed inflammatory cytokine expression, iNOS expression, and NO production as well as inhibited COX2 and therefore the generation of prostanoids [47]. These latter effects result from the ability of PPARγ to suppress proinflammatory genes through antagonism of the transcription factor NFκB, (and to a lesser extent, AP-1 and STATs) [8]. PPARγ agonists have also been demonstrated to suppress the Aβ-mediated activation of microglia in vitro and prevented cortical or hippocampal neuronal cell death [47–49]. In a rat model of cortical Aβ injection, coinjection of ciglitazone and ibuprofen or oral pioglitazone administration potently suppressed Aβ-evoked microglial cytokine generation. The effects of the PPARγ agonists pioglitazone and ibuprofen have been investigated in animal models of AD (Tg2576) that overexpress human APP. Pioglitazone was selected as it passes the blood brain barrier, although with limited penetration [50]. 12 months old Tg2576 mice were treated orally for 4 months resulting in a significant reduction of SDS-soluble Aβ40. Aβ42 levels were only significantly lowered for ibuprofen-treated animals, but a trend was observed for pioglitazone [51].

The modest effects of pioglitazone in this study were thought to be due to poor drug penetration into the brain. In a subsequent study treatment with larger doses of pioglitazone in aged APPV717I transgenic mice significantly decreased microglial and astroglial activation as well as Aβ plaque burden [52]. The finding that PPARγ agonists elicited a reduction in amyloid pathology may be the result of the ability of PPARγ to affect Aβ homeostasis. According to this hypothesis, evidence has been provided that immunostimulated β-site APP cleaving enzyme (BACE1) expression is silenced by a PPARγ-dependent regulation of the BACE1

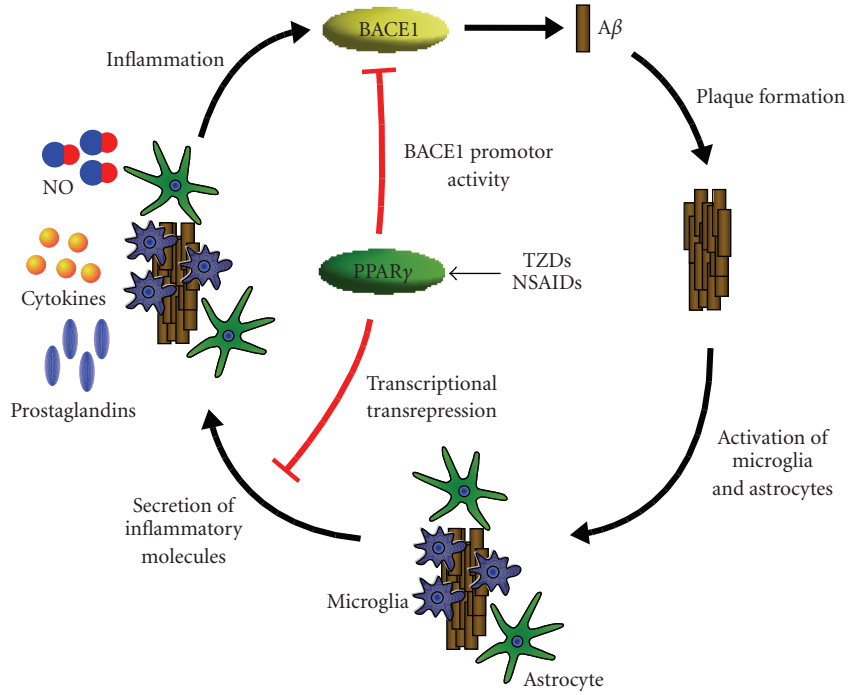

Figure 1: Effects of PPARγ on Aβ metabolism. Excessive production or insufficient clearance of Aβ results in its aggregation and finally in the formation of amyloid plaques. This process induces the activation of microglia as well as astrocytes which respond with the secretion of proinflammatory molecules like NO, cytokines, and prostaglandins developing the inflammatory phenotype of AD. In addition, cytokines are able to increase BACE1 activity thereby stimulating Aβ production. PPARγ agonists are able to abate both effects by either transrepress the production of proinflammatory molecules or directly interfere with the binding of PPARγ to a PPRE in the BACE1 gene promoter.

gene promoter [53, 54]. Similarly, oral pioglitazone treatment of APP transgenic mice reduced BACE1 transcription and expression. A recent study has found that PPARγ is associated with enhanced Aβ clearance. PPARγ activation, in both glia and neurons, led to a rapid and robust uptake and clearance of Aβ from the medium [55]. It has also been suggested that NSAIDs act directly on Aβ processing by the γ-secretase complex resulting in selective decrease of Aβ42 production [56, 57], even so this hypothesis has recently been challenged [58, 59].

Additionally, modulation of the Wnt/β-catenin signalling pathway may also account for some PPARγ-mediated beneficial effects in AD since recent findings show that PPARγ-mediated protection of hippocampal neurons against Aβ-induced toxicity directly correlates with β-catenin levels, inhibition of GSK-3β activity, and increased levels of Wnt-target genes [24, 60]. Furthermore, recent evidence suggests that PPARγ activation may also provide protection from excitotoxic stimuli [61] and positively influences neural stem cell proliferation and differentiation [62], both mechanisms that could potentially influence the overall salutary effects observed in models of neurodegenerative disease.

In a further animal study, Pedersen and colleagues have demonstrated that rosiglitazone treatment of Tg2576 mice resulted in behavioural improvement in these animals as well as in reduction of Aβ42 in the brain. Treatment with rosiglitazone for 34 months enhanced spatial working and reference memory [63]. Significantly, drug treatment was associated with a 25% reduction in Aβ1-42 levels, however Aβ1-40 levels remained unchanged. This reduction of Aβ1-42 was argued to arise from increased levels of insulin degrading enzyme (IDE) in rosiglitazone-treated transgenic mice, even so IDE has not been reported to be regulated by PPARγ. IDE is an Aβ degrading metalloprotease that has been genetically linked to AD [64].

The outcome of two clinical trials of the PPARγ agonist rosiglitazone has recently been reported [65, 66]. These studies reported that rosiglitazone therapy improves cognition in a subset of AD patients. Rosiglitazone does not pass the blood-brain barrier [65, 66], and this has been a confound in interpreting the CNS actions resulting from the administration of this drug. These data were interpreted as evidence for a significant role for peripheral insulin sensitivity in cognition. AD risk and memory impairment is associated with hyperinsulinemia, and insulin resistance, features which characterize type II diabetes [65, 67]. Indeed, type II diabetes is associated with increased risk of AD [67, 68]. Indeed, in a replication study PPARγ was found to be significantly associated with Alzheimer's disease [69]. Likewise, the Pro12Ala polymorphism within the exon 2 of PPARγ has been already linked to type 2 diabetes, insulin sensitivity, obesity, and cardiovascular diseases (for review see [70]). Even so the effect of this polymorphism is heterogeneous, since the Pro12Ala variant is associated with a reduced risk for diabetes [71–73], it has recently been shown that this polymorphism is associated with higher risk for Alzheimer's disease in octogenarians even after adjustment for the ApoE4 allele [74].

Clinical investigations of insulin-sensitizing TZDs that are in clinical use for type II diabetes are currently ongoing. A small study of 30 patients with mild AD or MCI found that 6 months of treatment with rosiglitazone resulted in improved memory and selective attention. A larger trial of rosiglitazone in AD patients has recently been reported [75]. More than 500 patients with mild to moderate AD were treated for 6 months with rosiglitazone, resulting in a statistically significant improvement in cognition in those patients that did not possess an ApoE4 allele [65]. Patients with ApoE4 did not respond to the drug and showed no improvement in standard cognitive tests. As an explanation it was suggested that rosiglitazone acts on mitochondria in the brain, increasing their metabolic efficiency and number. This hypothesis is supported by the observation that rosiglitazone induces neuronal mitochondrial DNA expression, enhances glucose utilization by inducing transcription of glucose metabolism and mitochondrial biogenesis genes leading to improved cellular function in mice. Noteworthy, these effects where also observed in animals expressing the ApoE4 allele. Determination of the amount of rosiglitazone in the brain revealed that 9–14% of the blood rosiglitazone crossed the blood brain barrier after oral treatment [76]. The actions of TZDs on mitochondria occur through both PPARγ-dependent and independent mechanisms [77]. The basis of the differential effects of rosiglitazone in individuals depending on their ApoE genotype is unexplained. The outcome of this clinical trial is, however, consistent with previous findings with respect to the influence of the ApoE4 genotype [78–80].

4. CONCLUSION

PPARs exhibit a wide range of activities to positively influence the pathology of Alzheimer's disease. Beside the ameliorating effect of PPARγ agonists on the inflammatory status of the AD brain by repressing the secretion of proinflammatory molecules and the enhancement of mitochondrial function, a direct involvement in the processing of the Aβ peptide has been demonstrated (Figure 1). The compelling results from animal models of Alzheimer's disease underline the beneficial effects of PPAR agonists for future therapies. The importance of these activities for the disease altering actions of PPAR agonist as well as the underlying molecular mechanisms have to be elucidated in ongoing and future research.

REFERENCES

[1] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[2] L. Michalik, J. Auwerx, J. P. Berger, et al., "International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors," *Pharmacological Reviews*, vol. 58, no. 4, pp. 726–741, 2006.

[3] T. M. Willson, M. H. Lambert, and S. A. Kliewer, "Peroxisome proliferator-activated receptor γ and metabolic disease," *Annual Review of Biochemistry*, vol. 70, pp. 341–367, 2001.

[4] G. D. Barish and R. M. Evans, "PPARs and LXRs: atherosclerosis goes nuclear," *Trends in Endocrinology and Metabolism*, vol. 15, no. 4, pp. 158–165, 2004.

[5] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[6] C. Diradourian, J. Girard, and J.-P. Pégorier, "Phosphorylation of PPARs: from molecular characterization to physiological relevance," *Biochimie*, vol. 87, no. 1, pp. 33–38, 2005.

[7] L. I. McKay and J. A. Cidlowski, "Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signaling pathways," *Endocrine Reviews*, vol. 20, no. 4, pp. 435–459, 1999.

[8] R. A. Daynes and D. C. Jones, "Emerging roles of PPARs in inflammation and immunity," *Nature Reviews Immunology*, vol. 2, no. 10, pp. 748–759, 2002.

[9] S. Ghisletti, W. Huang, S. Ogawa, et al., "Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARγ," *Molecular Cell*, vol. 25, no. 1, pp. 57–70, 2007.

[10] S. Ogawa, J. Lozach, C. Benner, et al., "Molecular determinants of crosstalk between nuclear receptors and toll-like receptors," *Cell*, vol. 122, no. 5, pp. 707–721, 2005.

[11] G. Krey, O. Braissant, F. L'Horset, et al., "Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay," *Molecular Endocrinology*, vol. 11, no. 6, pp. 779–791, 1997.

[12] A. Bernando and L. Minghetti, "PPAR-γ agonists as regulators of microglial activation and brain inflammation," *Current Pharmaceutical Design*, vol. 12, no. 1, pp. 93–109, 2006.

[13] J. M. Keller, P. Collet, A. Bianchi, et al., "Implications of peroxisome proliferator-activated receptors (PPARS) in development, cell life status and disease," *International Journal of Developmental Biology*, vol. 44, no. 5, pp. 429–442, 2000.

[14] R. Mukherjee, L. Jow, G. E. Croston, and J. R. Paterniti Jr., "Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists," *Journal of Biological Chemistry*, vol. 272, no. 12, pp. 8071–8076, 1997.

[15] D. Auboeuf, J. Rieusset, L. Fajas, et al., "Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients," *Diabetes*, vol. 46, no. 8, pp. 1319–1327, 1997.

[16] C. N. A. Palmer, M.-H. Hsu, K. J. Griffin, J. L. Raucy, and E. F. Johnson, "Peroxisome proliferator activated receptor-α expression in human liver," *Molecular Pharmacology*, vol. 53, no. 1, pp. 14–22, 1998.

[17] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[18] S. Moreno, S. Farioli-vecchioli, and M. P. Cerù, "Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS," *Neuroscience*, vol. 123, no. 1, pp. 131–145, 2004.

[19] J. W. Woods, M. Tanen, D. J. Figueroa, et al., "Localization of PPARδ in murine central nervous system: expression in oligodendrocytes and neurons," *Brain Research*, vol. 975, no. 1-2, pp. 10–21, 2003.

[20] F. Gofflot, N. Chartoire, L. Vasseur, et al., "Systematic gene expression mapping clusters nuclear receptors according to their function in the brain," *Cell*, vol. 131, no. 2, pp. 405–418, 2007.

[21] S. Basu-Modak, O. Braissant, P. Escher, B. Desvergne, P. Honegger, and W. Wahli, "Peroxisome proliferator-activated receptor β regulates acyl-CoA synthetase 2 in reaggregated rat brain cell cultures," *Journal of Biological Chemistry*, vol. 274, no. 50, pp. 35881–35888, 1999.

[22] A. Cimini, L. Cristiano, S. Colafarina, et al., "PPARγ-dependent effects of conjugated linoleic acid on the human glioblastoma cell line (ADF)," *International Journal of Cancer*, vol. 117, no. 6, pp. 923–933, 2005.

[23] M. T. Heneka, T. Klockgether, and D. L. Feinstein, "Peroxisome proliferator-activated receptor-γ ligands reduce neuronal inducible nitric oxide synthase expression and cell death in vivo," *The Journal of Neuroscience*, vol. 20, no. 18, pp. 6862–6867, 2000.

[24] N. C. Inestrosa, J. A. Godoy, R. A. Quintanilla, C. S. Koenig, and M. Bronfman, "Peroxisome proliferator-activated receptor γ is expressed in hippocampal neurons and its activation prevents β-amyloid neurodegeneration: role of Wnt signaling," *Experimental Cell Research*, vol. 304, no. 1, pp. 91–104, 2005.

[25] K. S. Park, R. D. Lee, S.-K. Kang, et al., "Neuronal differentiation of embryonic midbrain cells by upregulation of peroxisome proliferator-activated receptor-gamma via the JNK-dependent pathway," *Experimental Cell Research*, vol. 297, no. 2, pp. 424–433, 2004.

[26] S. A. Smith, G. R. Monteith, J. A. Robinson, N. G. Venkata, F. J. May, and S. J. Roberts-Thomson, "Effect of the peroxisome proliferator-activated receptor β activator GW0742 in rat cultured cerebellar granule neurons," *Journal of Neuroscience Research*, vol. 77, no. 2, pp. 240–249, 2004.

[27] A. Cimini, A. Bernardo, G. Cifone, L. Di Muzio, and S. Di Loreto, "TNFα downregulates PPARδ expression in oligodendrocyte progenitor cells: implications for demyelinating diseases," *Glia*, vol. 41, no. 1, pp. 3–14, 2003.

[28] I. Saluja, J. G. Granneman, and R. P. Skoff, "PPAR δ agonists stimulate oligodendrocyte differentiation in tissue culture," *Glia*, vol. 33, no. 3, pp. 191–204, 2001.

[29] L. Cristiano, A. Cimini, S. Moreno, A. M. Ragnelli, and M. P. Cerù, "Peroxisome proliferator-activated receptors (PPARs) and related transcription factors in differentiating astrocyte cultures," *Neuroscience*, vol. 131, no. 3, pp. 577–587, 2005.

[30] T. E. Cullingford, K. Bhakoo, S. Peuchen, C. T. Dolphin, R. Patel, and J. B. Clark, "Distribution of mRNAs encoding the peroxisome proliferator-activated receptor α, β, and γ and the retinoid X receptor α, β, and γ in rat central nervous system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1366–1375, 1998.

[31] S. Farioli-Vecchioli, S. Moreno, and M. P. Cerù, "Immunocytochemical localization of acyl-CoA oxidase in the rat central nervous system," *Journal of Neurocytology*, vol. 30, no. 1, pp. 21–33, 2001.

[32] R. E. Tanzi and L. Bertram, "Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective," *Cell*, vol. 120, no. 4, pp. 545–555, 2005.

[33] D. L. Price, R. E. Tanzi, D. R. Borchelt, and S. S. Sisodia, "Alzheimer's disease: genetic studies and transgenic models," *Annual Review of Genetics*, vol. 32, pp. 461–493, 1998.

[34] M. Hüll, K. Lieb, and B. L. Fiebich, "Pathways of inflammatory activation in Alzheimer's disease: potential targets for disease modifying drugs," *Current Medicinal Chemistry*, vol. 9, no. 1, pp. 83–88, 2002.

[35] H. Akiyama, S. Barger, S. Barnum, et al., "Inflammation and Alzheimer's disease," *Neurobiology of Aging*, vol. 21, no. 3, pp. 383–421, 2000.

[36] L. M. Sly, R. F. Krzesicki, J. R. Brashler, et al., "Endogenous brain cytokine mRNA and inflammatory responses to lipopolysaccharide are elevated in the Tg2576 transgenic mouse model of Alzheimer's disease," *Brain Research Bulletin*, vol. 56, no. 6, pp. 581–588, 2001.

[37] M. T. Heneka, H. Wiesinger, L. Dumitrescu-Ozimek, P. Riederer, D. L. Feinstein, and T. Klockgether, "Neuronal and glial coexpression of argininosuccinate synthetase and inducible nitric oxide synthase in Alzheimer disease," *Journal of Neuropathology & Experimental Neurology*, vol. 60, no. 9, pp. 906–916, 2001.

[38] S. C. Lee, M. Zhao, A. Hirano, and D. W. Dickson, "Inducible nitric oxide synthase immunoreactivity in the Alzheimer disease hippocampus: association with Hirano bodies, neurofibrillary tangles, and senile plaques," *Journal of Neuropathology & Experimental Neurology*, vol. 58, no. 11, pp. 1163–1169, 1999.

[39] Y. Vodovotz, M. S. Lucia, K. C. Flanders, et al., "Inducible nitric oxide synthase in tangle-bearing neurons of patients with Alzheimer's disease," *Journal of Experimental Medicine*, vol. 184, no. 4, pp. 1425–1433, 1996.

[40] S. M. de la Monte and J. R. Wands, "Molecular indices of oxidative stress and mitochondrial dysfunction occur early and often progress with severity of Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 9, no. 2, pp. 167–181, 2006.

[41] M. T. Heneka, G. E. Landreth, and D. L. Feinstein, "Role of peroxisome proliferator-activated receptor-γ in Alzheimer's disease," *Annals of Neurology*, vol. 49, no. 2, p. 276, 2001.

[42] T. Kielian and P. D. Drew, "Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation," *Journal of Neuroscience Research*, vol. 71, no. 3, pp. 315–325, 2003.

[43] G. E. Landreth and M. T. Heneka, "Anti-inflammatory actions of peroxisome proliferator-activated receptor gamma agonists in Alzheimer's disease," *Neurobiology of Aging*, vol. 22, no. 6, pp. 937–944, 2001.

[44] B. A. in 't Veld, A. Ruitenberg, A. Hofman, et al., "Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease," *The New England Journal of Medicine*, vol. 345, no. 21, pp. 1515–1521, 2001.

[45] J. M. Lehmann, J. M. Lenhard, B. B. Oliver, G. M. Ringold, and S. A. Kliewer, "Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs," *Journal of Biological Chemistry*, vol. 272, no. 6, pp. 3406–3410, 1997.

[46] M. T. Heneka, D. L. Feinstein, E. Galea, M. Gleichmann, U. Wüllner, and T. Klockgether, "Peroxisome proliferator-activated receptor gamma agonists protect cerebellar granule cells from cytokine-induced apoptotic cell death by inhibition of inducible nitric oxide synthase," *Journal of Neuroimmunology*, vol. 100, no. 1-2, pp. 156–168, 1999.

[47] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of β- amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists," *The Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[48] E. J. Kim, K. J. Kwon, J.-Y. Park, S. H. Lee, C.-H. Moon, and E. J. Baik, "Effects of peroxisome proliferator-activated receptor agonists on LPS-induced neuronal death in mixed cortical neurons: associated with iNOS and COX-2," *Brain Research*, vol. 941, no. 1-2, pp. 1–10, 2002.

[49] R. Luna-Medina, M. Cortes-Canteli, M. Alonso, A. Santos, A. Martínez, and A. Perez-Castillo, "Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor γ activation," *Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21453–21462, 2005.

[50] Y. Maeshiba, Y. Kiyota, K. Yamashita, Y. Yoshimura, M. Motohashi, and S. Tanayama, "Disposition of the new antidiabetic agent pioglitazone in rats, dogs, and monkeys," *Arzneimittel-Forschung*, vol. 47, no. 1, pp. 29–35, 1997.

[51] Q. Yan, J. Zhang, H. Liu, et al., "Anti-inflammatory drug therapy alters β-amyloid processing and deposition in an animal model of Alzheimer's disease," *The Journal of Neuroscience*, vol. 23, no. 20, pp. 7504–7509, 2003.

[52] M. T. Heneka, M. Sastre, L. Dumitrescu-Ozimek, et al., "Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice," *Journal of Neuroinflammation*, vol. 2, article 22, 2005.

[53] M. Sastre, T. Klockgether, and M. T. Heneka, "Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms," *International Journal of Developmental Neuroscience*, vol. 24, no. 2-3, pp. 167–176, 2006.

[54] M. Sastre, I. Dewachter, G. E. Landreth, et al., "Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-γ agonists modulate immunostimulated processing of amyloid precursor protein through regulation of β-secretase," *The Journal of Neuroscience*, vol. 23, no. 30, pp. 9796–9804, 2003.

[55] I. E. Camacho, L. Serneels, K. Spittaels, P. Merchiers, D. Dominguez, and B. De Strooper, "Peroxisome proliferator-activated receptor γ induces a clearance mechanism for the amyloid-β peptide," *The Journal of Neuroscience*, vol. 24, no. 48, pp. 10908–10917, 2004.

[56] J. L. Eriksen, S. A. Sagi, T. E. Smith, et al., "NSAIDs and enantiomers of flurbiprofen target γ-secretase and lower Aβ42 in vivo," *The Journal of Clinical Investigation*, vol. 112, no. 3, pp. 440–449, 2003.

[57] S. Weggen, J. L. Eriksen, P. Das, et al., "A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity," *Nature*, vol. 414, no. 6860, pp. 212–216, 2001.

[58] T. Morihara, B. Teter, F. Yang, et al., "Ibuprofen suppresses interleukin-1β induction of pro-amyloidogenic α_1-antichymotrypsin to ameliorate β-amyloid (Aβ) pathology in Alzheimer's models," *Neuropsychopharmacology*, vol. 30, no. 6, pp. 1111–1120, 2005.

[59] T. A. Lanz, G. J. Fici, and K. M. Merchant, "Lack of specific amyloid-β(1-42) suppression by nonsteroidal anti-inflammatory drugs in young, plaque-free Tg2576 mice and in guinea pig neuronal cultures," *Journal of Pharmacology and Experimental Therapeutics*, vol. 312, no. 1, pp. 399–406, 2005.

[60] R. A. Fuentealba, G. Farias, J. Scheu, M. Bronfman, M. P. Marzolo, and N. C. Inestrosa, "Signal transduction during amyloid-β-peptide neurotoxicity: role in Alzheimer disease," *Brain Research Reviews*, vol. 47, no. 1–3, pp. 275–289, 2004.

[61] X. Zhao, Z. Ou, J. C. Grotta, N. Waxham, and J. Aronowski, "Peroxisome-proliferator-activated receptor-gamma (PPARγ) activation protects neurons from NMDA excitotoxicity," *Brain Research*, vol. 1073-1074, pp. 460–469, 2006.

[62] K. Wada, A. Nakajima, K. Katayama, et al., "Peroxisome proliferator-activated receptor γ-mediated regulation of neural stem cell proliferation and differentiation," *Journal of Biological Chemistry*, vol. 281, no. 18, pp. 12673–12681, 2006.

[63] W. A. Pedersen, P. J. McMillan, J. J. Kulstad, J. B. Leverenz, S. Craft, and G. R. Haynatzki, "Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice," *Experimental Neurology*, vol. 199, no. 2, pp. 265–273, 2006.

[64] W. Q. Qiu and M. F. Folstein, "Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer's disease: review and hypothesis," *Neurobiology of Aging*, vol. 27, no. 2, pp. 190–198, 2006.

[65] M. E. Risner, A. M. Saunders, J. F. B. Altman, et al., "Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease," *The Pharmacogenomics Journal*, vol. 6, no. 4, pp. 246–254, 2006.

[66] M. Chalimoniuk, K. King-Pospisil, W. A. Pedersen, et al., "Arachidonic acid increases choline acetyltransferase activity in spinal cord neurons through a protein kinase C-mediated mechanism," *Journal of Neurochemistry*, vol. 90, no. 3, pp. 629–636, 2004.

[67] J. A. Luchsinger, M.-X. Tang, Y. Stern, S. Shea, and R. Mayeux, "Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort," *American Journal of Epidemiology*, vol. 154, no. 7, pp. 635–641, 2001.

[68] R. Peila, B. L. Rodriguez, and L. J. Launer, "Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia Aging Study," *Diabetes*, vol. 51, no. 4, pp. 1256–1262, 2002.

[69] G. Hamilton, P. Proitsi, L. Jehu, et al., "Candidate gene association study of insulin signaling genes and Alzheimer's disease: evidence for *SOS2*, *PCK1*, and PPARγ as susceptibility loci," *American Journal of Medical Genetics, Part B*, vol. 144, no. 4, pp. 508–516, 2007.

[70] M. Stumvoll and H. Häring, "The peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism," *Diabetes*, vol. 51, no. 8, pp. 2341–2347, 2002.

[71] D. Altshuler, J. N. Hirschhorn, M. Klannemark, et al., "The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes," *Nature Genetics*, vol. 26, no. 1, pp. 76–80, 2000.

[72] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[73] L. Frederiksen, K. Brødbæk, M. Fenger, et al., "Studies of the Pro12Ala polymorphism of the PPAR-γ gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 8, pp. 3989–3992, 2002.

[74] R. Scacchi, A. Pinto, G. Gambina, A. Rosano, and R. M. Corbo, "The peroxisome proliferator-activated receptor gamma (PPAR-γ2) Pro12Ala polymorphism is associated with higher risk for Alzheimer's disease in octogenarians," *Brain Research*, vol. 1139, pp. 1–5, 2007.

[75] G. S. Watson, B. A. Cholerton, M. A. Reger, et al., "Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study," *American Journal of Geriatric Psychiatry*, vol. 13, no. 11, pp. 950–958, 2005.

[76] J. C. Strum, R. Shehee, D. Virley, et al., "Rosiglitazone induces mitochondrial biogenesis in mouse brain," *Journal of Alzheimer's Disease*, vol. 11, no. 1, pp. 45–51, 2007.

[77] D. L. Feinstein, A. Spagnolo, C. Akar, et al., "Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key?" *Biochemical Pharmacology*, vol. 70, no. 2, pp. 177–188, 2005.

[78] S. Craft, S. Asthana, G. Schellenberg, et al., "Insulin metabolism in Alzheimer's disease differs according to the

apolipoprotein E genotype and gender," *Neuroendocrinology*, vol. 70, no. 2, pp. 146–152, 1999.

[79] S. Craft, S. Asthana, G. Schellenberg, et al., "Insulin effects on glucose metabolism, memory, and plasma amyloid precursor protein in Alzheimer's disease differ according to apolipoprotein-E genotype," *Annals of the New York Academy of Sciences*, vol. 903, pp. 222–228, 2000.

[80] J. Kuusisto, K. Koivisto, L. Mykkänen, et al., "Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: cross sectional population based study," *British Medical Journal*, vol. 315, no. 7115, pp. 1045–1049, 1997.

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Review Article

Potential Therapeutic Targets for PPARγ after Spinal Cord Injury

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Traumatic injury to the spinal cord results in multiple anatomical, physiological, and functional deficits as a result of local neuronal and glial cell death as well as loss of descending and ascending axons traversing the injury site. The many different mechanisms thought to contribute to protracted secondary cell death and dysfunction after spinal cord injury (SCI) are potential therapeutic targets. Agents that bind and activate the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) show great promise for minimizing or preventing these deleterious cascades in other models of CNS disorders. This review will summarize the major secondary injury cascades occurring after SCI and discuss data from experimental CNS injury and disease models showing the exciting potential for PPARγ therapies after SCI.

1. WHAT IS PPARγ?

"PPAR" is an acronym for peroxisome proliferator-activated receptor, which refers to a family of nuclear hormone receptors that function as ligand-activated transcription factors. Three PPAR isoforms have been identified to date, including PPARα, PPARβ/δ, and PPARγ (for review, see [1–3]). Upon activation, PPAR molecules heterodimerize with retinoid X receptors (RXRs), which are the nuclear receptors for 9-*cis* retinoic acid. After dimerization, the PPAR/RXR complex binds to specific promoter sequences on target genes where it can promote or repress gene activation. Most initial research on these molecules focused on their role in lipid metabolism and homeostasis [1, 4]. However, it is now known that PPARs function in a much broader array of physiological functions, both under normal conditions and following injury or disease. In particular, upregulation of PPARγ mRNA has been detected in inflammatory cells and in experimental models of CNS injury such as ischemic stroke [5, 6]. PPARγ agonists appear to have potent anti-inflammatory and neuroprotective actions [7–9]; thus, this transcription factor may be involved in coordinating cellular responses to CNS injury. This also presents the opportunity to enhance neuroprotection by leveraging PPARγ expression through administration of specific agonists following CNS damage. Indeed, over the past decade, several studies have revealed beneficial actions of promoting PPARγ activation in experimental models of CNS injury, ischemia, and disease. Less work has examined the potential of promoting PPARγ activation following injury to the spinal cord, for which current clinical therapies are limited. This review will summarize the documented beneficial actions of PPARγ following CNS injury and illustrate how they may also promote anatomical and behavioral recovery after spinal cord injury (SCI).

2. SPINAL CORD INJURY: THE FACTS

In the United States, a new SCI is sustained on average every 41 minutes, which results in ~1100 new cases each year. The majority of these injuries are caused by motor vehicle accidents, followed by accidents such as falling from ladders or diving into shallow water [10]. Most SCI's occur in young individuals, particularly males—in their late teens or twenties. Because medical care has improved dramatically during the previous century, most individuals can expect to live many years following an SCI. Their lives, however, are not easy and they have many issues with which to deal on a daily basis. In the eyes of most uninjured people, the most obvious problem affecting SCI individuals is their inability to walk. While this is clearly a significant obstacle to overcome, a recent survey

of paraplegics and quadriplegics revealed that regaining locomotor function is actually of lesser importance to them compared to the many other issues they face [11]. For instance, quadriplegics would prefer restoration of hand and arm function over walking. Paraplegics' top choice would be regaining normal sexual function, an important issue in terms of relationships with significant others, and the ability to have a family. Also high on the list for all spinal-injured people was return of bowel and bladder function. Other serious issues many face include potentially fatal autonomic dysreflexia, pressure sores that can take several months to heal, and untreatable intractable pain [10].

Since it is impossible to prevent the occurrence of most SCIs, our best hope is to improve the level of recovery achievable after an SCI occurs. Most SCIs result from a contusion-type injury in which the vertebral bodies and/or intervertebral discs are rapidly displaced into the spinal canal causing crushing and bruising of the delicate spinal tissue [12, 13]. The initial impact leads to immediate hemorrhage and rapid cell death at the impact site. This is followed by multiple secondary injury cascades that cause further tissue loss and dysfunction [10, 14]. If these secondary injury processes were minimized or eliminated, the outcome for patients would be greatly improved. Many of these cascades are potential targets for intervention by activation of the transcription factor PPARγ. Indeed, two recent studies demonstrated that treatment of SCI rodents with a PPARγ agonist results in significantly improved anatomical sparing and locomotor abilities [15, 16]. The rest of this review will discuss specific secondary injury processes that occur after SCI and how PPARγ activation may be used to lessen their impact.

3. GLUTAMATE EXCITOTOXICITY: A GOOD TRANSMITTER GONE BAD

Within minutes of SCI, extracellular glutamate levels rise within and around the injury site [17]. This potent neurotransmitter can then diffuse to surviving cells, bind to surface receptors, and lead to what is known as excitotoxic cell death [18]. Especially vulnerable to excitotoxicity are neurons and oligodendrocytes, which express a full complement of glutamate receptors. Loss of neurons at the injury site will lead to direct denervation and paralysis of muscle fibers innervated by those neurons, thereby contributing to motor deficits. Because a significant amount of sensory processing occurs within the spinal cord, especially that involved in pain and temperature sensation, loss of neurons can also lead to hypersensitivity, paresthesia, enhanced and prolonged pain, and/or total loss of pain and temperature sensation. Excitotoxic injury to oligodendrocytes can result in demyelination of axons around the injury site. This, in turn, will lead to a drastic reduction or complete halt of axonal transmission, thereby enhancing the disconnection between the brain and spinal segments below the level of injury. Thus, excitotoxicity has the potential to markedly exacerbate the functional problems encountered after SCI. Indeed, the involvement of excess glutamate in cell death after SCI was demonstrated by studies in which early treatment with glutamate antagonists significantly enhanced tissue preservation and functional recovery following SCI in rats [19, 20].

A major mechanism responsible for maintaining low extracellular glutamate levels is astrocytic uptake via glutamate transporters, including GLT1/EAAT2 which is responsible for removal of up to 90% of extracellular glutamate. While glutamate transporters are effective under basal conditions, they become saturated when glutamate levels rise substantially above normal. Thus, a mechanism for increasing expression of GLT1/EAAT2 and other glutamate transporters could be highly beneficial after SCI. Recent work reveals that PPARγ activation may do just that. Using a cell culture model of ischemic preconditioning, Romera et al. [21] showed that preconditioning upregulates PPARγ expression in neurons and astrocytes, and that treatment of the cultures with a PPARγ agonist significantly increased astrocytic expression of GLT1/EAAT2 mRNA and protein. They also showed that this increased expression translated into enhanced glutamate uptake and reduced cell death. The proposed mechanism was a direct increase in EAAT2 promoter activity induced by activated PPARγ. A direct neuroprotective action by PPARγ activation under excitotoxic conditions has also been demonstrated using cultures of pure cortical neurons [22]. In vivo evidence supports the notion that PPARγ activation is protective against glutamate excitotoxicity. For instance, treatment with a PPARγ agonist decreased neuron loss caused by intracortical injection of a glutamate receptor agonist [22]. While changes in glutamate levels in SCI models treated with PPARγ agonists have not yet been measured, protection against glutamate excitotoxicity is a plausible mechanism by which PPARγ could improve outcome after SCI.

4. LIPID PEROXIDATION

A well-documented pathological process occurring early after SCI is the formation of reactive oxygen and nitrogen species (ROS and RNS, resp.); this results from increased intracellular calcium levels, mitochondrial dysfunction, arachidonic acid breakdown, and activation of inducible nitric oxide synthase (iNOS) [23–25]. Initially thought to be a problem only in acute SCI tissue, newer studies have revealed that indices of free radical damage are present throughout the first week after injury [26, 27]. ROS and RNS cause lipid peroxidation as well as oxidative and nitrative damage to proteins and nucleic acids [27, 28]. In addition, oxidative damage exacerbates mitochondrial dysfunction [29] and contributes to intracellular calcium overload which activates proteases resulting in breakdown of cytoskeletal proteins [27, 30]. Thus, the collective damage induced by ROS and RNS is far-reaching and likely contributes to cellular death and functional loss after SCI.

PPARγ activation after SCI could dampen the damage induced by ROS and RNS in multiple ways. First, PPARγ activation may reduce the overall level of free radicals present in the injured tissue since PPARγ activation leads to decreased nitric oxide, cyclooxygenase-2 (COX-2), iNOS, and nitrotyrosine levels in animal models of amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), ischemia, and neuroinflammation [31–37]. In addition, PPARγ agonists may

increase the levels of antioxidants in or around the injured tissue. For instance, catalase levels were elevated by PPARγ agonist treatment following intracerebral hemorrhage [38]; with increased antioxidant levels, the surviving tissue will be better equipped to fend off assault by free radicals. Thus, agonists that stimulate PPARγ may reduce the levels of free radicals and at the same time, elevate enzymes essential for combating free radicals that remain. This in turn would reduce the number of neurons and glial cells that die in the subacute phase of SCI. Studies by our group and others have shown that treatment with the PPARγ agonist pioglitazone resulted in an increase in the number of motor neurons spared after SCI, which might have been due, at least in part, to a reduction in post-SCI oxidative damage [15, 16]. By promoting motor neuron survival in human SCI, significant preservation of segmental function may be possible. Although complete recovery of "normal" function may not be feasible, even partial recovery of hand function, for instance, could drastically improve the quality of life for an individual with SCI.

5. INFLAMMATORY-MEDIATED CELL DEATH

A well-characterized event after spinal trauma is local microglial activation, inflammatory cell infiltration, and upregulation of proinflammatory mediators. Indeed, several studies have shown the rapid rise in proinflammatory cytokines and chemokines which stimulate inflammatory cell infiltration into the injured spinal cord [14]. Once present within the damaged and surrounding parenchyma, inflammatory cells such as neutrophils, macrophages, and lymphocytes can exacerbate tissue damage. For instance, activated macrophages and microglia produce cytotoxic molecules such as iNOS, TNFα, IL-1β, and IL-6. Interestingly, PPARγ levels are upregulated in activated microglia and macrophages [35, 39, 40] and activation of PPARγ in these cells can decrease production of proinflammatory mediators [40–42]. The mechanisms contributing to these effects include antagonism of AP-1, STAT, and NFκB levels as well as concomitant increases in IκBα levels. Collectively, these actions will reduce the inflammatory potential of the treated cells [37, 38, 40, 43]. Accordingly, PPAR-induced inhibition of microglia/macrophage accumulation and release of proinflammatory cytokines has been detected in animal models of Alzheimer's disease, Parkinson's disease, and MS [33, 34, 44–46]. PPARγ activation can also reduce the differentiation of monocytes into macrophages [45], promote macrophage apoptosis [47], and decrease T-cell proliferation, which collectively would result in reduced numbers of infiltrating inflammatory cells into the injured spinal cord [48]. Indeed, PPARγ-induced reductions in neutrophil, T-cell, and macrophage infiltration have been shown in animal studies of experimental allergic encephalomyelitis (EAE, an animal model of MS) and intracerebral hemorrhage [33, 38, 48]. This may be due, in part to, a PPARγ-mediated reduction in chemokines, which elicit inflammatory cell recruitment to the CNS. For instance, mice with EAE given oral PPARγ agonists expressed lower levels of MIP1α and RANTES within the brain compared to control mice [33].

Collectively, these data suggest that PPARγ activation provides a potent means for reducing proinflammatory mediators after CNS injury, including trauma to the spinal cord. This is further suggested by recent SCI studies which revealed a reduction in gliosis, cytokines, and adhesion molecules [16]. Many SCI studies have demonstrated that postinjury treatment with anti-inflammatory agents results in significantly improved anatomical and functional recovery [49–54]. Thus, the anti-inflammatory actions of activating the PPARγ pathway could provide another mechanism for reducing the deleterious proinflammatory cascades initiated after SCI.

6. DEMYELINATION OF SURVIVING AXONS AFTER SCI

Another pathological feature of acute and chronic SCI tissue is demyelination of axons that survive the initial traumatic event [55–57]. Loss of myelin will lead to conduction delays and/or frank conduction block. Because axons traversing the injury site are the sole remaining connection between the brain and caudal spinal neurons, inefficient communication through these axons is a significant clinical issue. Demyelination is due to loss of oligodendrocytes, which are killed at the injury epicenter within hours of the injury and continue to undergo apoptosis in rostral and caudal white matter for many weeks after SCI [58–60]. The potential mechanisms responsible for acute and delayed oligodendrocyte death are numerous. For instance, oligodendrocytes are known to be susceptible to glutamate excitotoxicity, which could contribute to the early loss of these cells. Oligodendrocytes and their myelin membranes are also vulnerable to lipid peroxidation, which, as stated above, is prevalent throughout the first week after injury. Lastly, proinflammatory mediators such as TNFα and IL-1β can lead to oligodendrocyte death.

Since PPARγ activation can counteract many of these deleterious processes, this pathway may thereby promote oligodendrocyte survival and myelin preservation following CNS damage. Indeed, treatment with a PPARγ agonist markedly improved myelination and decreased lesion area in the CNS of animals with EAE [33, 44, 48, 61]. In addition, a PPARγ agonist was able to reduce myelin damage in an in vitro model of inflammatory demyelination [62]. Therefore, treatment with PPARγ agonists after SCI could potentially lead to improved oligodendrocyte survival and better myelin preservation. Indeed, we have noted that when the PPARγ agonist pioglitazone was given to rats after SCI, a significant increase in sparing of white matter distal to the lesions was detected [15]. This likely contributed to the improved locomotor function detected in our study and others [15, 16]. Thus, acute treatment of SCI patients with a PPARγ agonist could potentially improve tissue sparing and thereby allow for a greater level of locomotor abilities as well as other important functional outcomes. For example, neurons controlling bowel and bladder function are located within the lower spinal cord, including the lumbar and sacral segments. Because most SCI's occur in more rostral segments, neuronal circuits that directly control bowel and bladder are often intact after SCI. However, significant and permanent bowel and bladder dysfunction occurs due to loss of descending

Table 1: Summary of effects mediated by PPARγ activation in CNS injury or disease models.

Disease/injury model	Known PPARγ effects	References
Spinal cord injury	↑locomotor recovery ↑myelin sparing ↑motor neuron sparing ↓glial activation ↓proinflammatory cytokines	[15, 16]
Experimental allergic encephalomyelitis *(model of multiple sclerosis)*	↑myelin sparing ↓lesion size ↓inflammatory cell infiltrate ↓proinflammatory cytokines & chemokines ↓clinical score (better recovery; lower no. of relapses)	[33, 44, 48, 61]
Amyotrophic lateral sclerosis	↑motor neuron survival ↓glial activation ↓ COX-2 ↓ iNOS ↑longevity, delayed disease onset	[32]
Parkinson's disease	↑dopaminergic neuron survival ↓gliosis ↓ iNOS ↓ NFκB translocation to the nucleus ↑ IκBα	[43, 46]
Cerebral ischemia	↑neuron survival in penumbra ↓ lesion size ↓COX-2 ↓ proinflammatory cytokines ↑ antioxidants	[6, 31, 35, 37, 64]
Cerebral hemorrhage	↑ catalase ↓NFκB ↓ neutrophil infiltration ↓ apoptosis ↓ behavioral dysfunction	[38]
Alzheimer's disease	↓gliosis ↓COX-2 & iNOS ↓proinflammatory cytokines ↓Aβ1-42+ amyloid plaques ↓β-secretase mRNA ↓monocyte differentiation into macrophages	[7, 45, 65, 66]

signals carried by axons that are lost or demyelinated at the impact site. Therefore, enhanced preservation of myelin or promotion of remyelination at the injury site could lead to functionally significant improvements in the quality of life for SCI patients.

7. NEURON LOSS AFTER SCI

Contusion-type injuries are the most commonly sustained trauma to the spinal cord. Because of the high degree of vascularization and the dynamic forces encountered within the gray matter during contusive injuries [63], the lesions evolve as centralized fluid-filled cavities originating within gray matter regions at the lesion site that extend into rostral and caudal segments. Thus, even mild contusions can result in significant neuron loss over multiple segments of spinal cord. As stated above, neurons not killed by the initial impact can fall victim to secondary cascades, including ischemia, excitotoxicity, lipid peroxidation, and proinflammatory mediators. This neuron death will lead directly to loss of function in the muscles innervated by motor neurons at the segment of injury. Since the majority of injuries occur in the cervical spinal cord, SCI often means loss of function in the arms and hands. In addition, motor neurons driving respiration are found within C3–C5, so injuries that directly damage these segments frequently result in respirator dependence.

Clearly, therapies that protect neurons from secondary injury cascades after SCI are of great importance. Given the potential beneficial actions of PPARγ activation discussed above, neuroprotection after SCI is an important potential therapeutic target for PPARγ agonists. Indeed, improved neuronal survival following PPARγ agonist treatment has already been noted in several models of CNS disorders. For instance, treatment with the PPARγ agonist pioglitazone promoted motor neuron survival and increased muscle fiber diameter in a transgenic model of ALS [32]. PPARγ agonists also increased neuron survival and decreased lesion sizes in models of Parkinson's disease [43, 46], central inflammation [34], intracerebral hemorrhage [38], and cerebral ischemia [5, 6, 31, 35]. These beneficial effects were likely mediated through a reduction in the indirect actions noted above, including lipid peroxidation, proinflammatory signals, and extracellular glutamate levels. However, PPARγ activation may also have a direct effect on neurons. Neuronal expression of PPARγ has been detected in the intact CNS and an upregulation of PPARγ was observed in neurons in the ischemic penumbra following focal cerebral ischemia [5, 6, 31, 35]. Furthermore, cultured neurons treated with PPARγ agonists were protected from glutamate-induced death demonstrating a direct action of PPARγ activation in neurons [22]. Thus, if a PPARγ agonist was delivered soon after SCI, significant neuronal sparing may be achieved which would likely translate into better functional preservation and improved quality of life for SCI patients.

8. SUMMARY

The PPAR pathway appears to play an important role in recovery from CNS disorders (Table 1). Indeed, several studies suggest that endogenous ligands present in the damaged CNS can activate the PPARγ pathway and contribute to anatomical preservation. This is illustrated by studies demonstrating that PPARγ antagonists potentiate tissue pathology after cerebral ischemia [5, 6]. Exacerbation of neuropathology also occurs when EAE is induced in the presence of a PPARγ antagonist or when disease is induced in PPARγ knockout mice [61, 67]. Thus, endogenous PPARγ activation may be an essential component of promoting spontaneous reparative mechanisms that are initiated in the injured brain and spinal cord. This endogenous response appears submaximal, however, as the numerous studies discussed above suggest that pharmacological activation of the PPARγ pathway subsequent to damage may significantly improve recovery. In the realm of SCI research, treatments are severely lacking. Therefore, manipulating the PPARγ pathway appears to hold great potential as a therapy for treating human SCI.

REFERENCES

[1] A. K. Hihi, L. Michalik, and W. Wahli, "PPARs: transcriptional effectors of fatty acids and their derivatives," *Cellular and Molecular Life Sciences*, vol. 59, no. 5, pp. 790–798, 2002.

[2] C. Blanquart, O. Barbier, J. C. Fruchart, B. Staels, and C. Glineur, "Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 85, no. 2–5, pp. 267–273, 2003.

[3] R. Bordet, T. Ouk, O. Petrault, et al., "PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases," *Biochemical Society Transactions*, vol. 34, no. 6, pp. 1341–1346, 2006.

[4] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[5] N. A. Victor, E. W. Wanderi, J. Gamboa, et al., "Altered PPARγ expression and activation after transient focal ischemia in rats," *European Journal of Neuroscience*, vol. 24, no. 6, pp. 1653–1663, 2006.

[6] Z. Ou, X. Zhao, L. A. Labiche, et al., "Neuronal expression of peroxisome proliferator-activated receptor-γ (PPARγ) and 15d-prostaglandin J_2-mediated protection of brain after experimental cerebral ischemia in rat," *Brain Research*, vol. 1096, no. 1, pp. 196–203, 2006.

[7] G. E. Landreth and M. T. Heneka, "Anti-inflammatory actions of peroxisome proliferator-activated receptor γ agonists in Alzheimer's disease," *Neurobiology of Aging*, vol. 22, no. 6, pp. 937–944, 2001.

[8] R. Genolet, W. Wahli, and L. Michalik, "PPARs as drug targets to modulate inflammatory responses?" *Current Drug Targets—Inflammation & Allergy*, vol. 3, no. 4, pp. 361–375, 2004.

[9] A. Bernardo and L. Minghetti, "PPAR-γ agonists as regulators of microglial activation and brain inflammation," *Current Pharmaceutical Design*, vol. 12, no. 1, pp. 93–109, 2006.

[10] L. H. Sekhon and M. G. Fehlings, "Epidemiology, demographics, and pathophysiology of acute spinal cord injury," *Spine*, vol. 26, supplement 24, pp. 2–12, 2001.

[11] K. D. Anderson, "Targeting recovery: priorities of the spinal cord-injured population," *Journal of Neurotrauma*, vol. 21, no. 10, pp. 1371–1383, 2004.

[12] R. P. Bunge, W. R. Puckett, J. L. Becerra, A. Marcillo, and R. M. Quencer, "Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination," *Advances in Neurology*, vol. 59, pp. 75–89, 1993.

[13] B. A. Kakulas, "A review of the neuropathology of human spinal cord injury with emphasis on special features," *Journal of Spinal Cord Medicine*, vol. 22, no. 2, pp. 119–124, 1999.

[14] C. Profyris, S. S. Cheema, D. Zang, M. F. Azari, K. Boyle, and S. Petratos, "Degenerative and regenerative mechanisms governing spinal cord injury," *Neurobiology of Disease*, vol. 15, no. 3, pp. 415–436, 2004.

[15] D. M. McTigue, R. Tripathi, P. Wei, and A. T. Lash, "The PPAR γ agonist Pioglitazone improves anatomical and locomotor recovery after rodent spinal cord injury," *Experimental Neurology*, vol. 205, no. 2, pp. 396–406, 2007.

[16] S.-W. Park, J.-H. Yi, G. Miranpuri, et al., "Thiazolidinedione class of peroxisome proliferator-activated receptor γ agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1002–1012, 2007.

[17] D. J. McAdoo, G.-Y. Xu, G. Robak, and M. G. Hughes, "Changes in amino acid concentrations over time and space around an impact injury and their diffusion through the rat spinal cord," *Experimental Neurology*, vol. 159, no. 2, pp. 538–544, 1999.

[18] G.-Y. Xu, M. G. Hughes, L. Zhang, L. Cain, and D. J. McAdoo, "Administration of glutamate into the spinal cord at extracellular concentrations reached post-injury causes functional impairments," *Neuroscience Letters*, vol. 384, no. 3, pp. 271–276, 2005.

[19] L. J. Rosenberg, Y. D. Teng, and J. R. Wrathall, "2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline reduces glial loss and acute white matter pathology after experimental spinal cord contusion," *Journal of Neuroscience*, vol. 19, no. 1, pp. 464–475, 1999.

[20] J. R. Wrathall, Y. D. Teng, and R. Marriott, "Delayed antagonism of AMPA/kainate receptors reduces long-term functional deficits resulting from spinal cord trauma," *Experimental Neurology*, vol. 145, no. 2, pp. 565–573, 1997.

[21] C. Romera, O. Hurtado, J. Mallolas, et al., "Ischemic preconditioning reveals that GLT1/EAAT2 glutamate transporter is a novel PPARγ target gene involved in neuroprotection," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 7, pp. 1327–1338, 2007.

[22] X. Zhao, Z. Ou, J. C. Grotta, N. Waxham, and J. Aronowski, "Peroxisome-proliferator-activated receptor-γ (PPARγ) activation protects neurons from NMDA excitotoxicity," *Brain Research*, vol. 1073-1074, no. 1, pp. 460–469, 2006.

[23] E. D. Hall and J. E. Springer, "Neuroprotection and acute spinal cord injury: a reappraisal," *NeuroRX*, vol. 1, no. 1, pp. 80–100, 2004.

[24] J. E. Springer, R. D. Azbill, R. J. Mark, J. G. Begley, G. Waeg, and M. P. Mattson, "4-hydroxynonenal, a lipid peroxidation product, rapidly accumulates following traumatic spinal cord injury and inhibits glutamate uptake," *Journal of Neurochemistry*, vol. 68, no. 6, pp. 2469–2476, 1997.

[25] R. D. Azbill, X. Mu, A. J. Bruce-Keller, M. P. Mattson, and J. E. Springer, "Impaired mitochondrial function, oxidative stress and altered antioxidant enzyme activities following traumatic spinal cord injury," *Brain Research*, vol. 765, no. 2, pp. 283–290, 1997.

[26] J. Luo, K. Uchida, and R. Shi, "Accumulation of acrolein-protein adducts after traumatic spinal cord injury," *Neurochemical Research*, vol. 30, no. 3, pp. 291–295, 2005.

[27] Y. Xiong, A. G. Rabchevsky, and E. D. Hall, "Role of peroxynitrite in secondary oxidative damage after spinal cord injury," *Journal of Neurochemistry*, vol. 100, no. 3, pp. 639–649, 2007.

[28] W. Xu, L. Chi, R. Xu, et al., "Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury," *Spinal Cord*, vol. 43, no. 4, pp. 204–213, 2005.

[29] P. G. Sullivan, S. Krishnamurthy, S. P. Patel, J. D. Pandya, and A. G. Rabchevsky, "Temporal characterization of mitochondrial bioenergetics after spinal cord injury," *Journal of Neurotrauma*, vol. 24, no. 6, pp. 991–999, 2007.

[30] J. M. Wingrave, K. E. Schaecher, E. A. Sribnick, et al., "Early induction of secondary injury factors causing activation of calpain and mitochondria-mediated neuronal apoptosis following spinal cord injury in rats," *Journal of Neuroscience Research*, vol. 73, no. 1, pp. 95–104, 2003.

[31] J. Culman, Y. Zhao, P. Gohlke, and T. Herdegen, "PPAR-γ: therapeutic target for ischemic stroke," *Trends in Pharmacological Sciences*, vol. 28, no. 5, pp. 244–249, 2007.

[32] B. Schütz, J. Reimann, L. Dumitrescu-Ozimek, et al., "The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice," *Journal of Neuroscience*, vol. 25, no. 34, pp. 7805–7812, 2005.

[33] D. L. Feinstein, E. Galea, V. Gavrilyuk, et al., "Peroxisome proliferator-activated receptor-γ agonists prevent experimental autoimmune encephalomyelitis," *Annals of Neurology*, vol. 51, no. 6, pp. 694–702, 2002.

[34] M. T. Heneka, T. Klockgether, and D. L. Feinstein, "Peroxisome proliferator-activated receptor-γ ligands reduce neuronal inducible nitric oxide synthase expression and cell death *in vivo* ," *Journal of Neuroscience*, vol. 20, no. 18, pp. 6862–6867, 2000.

[35] Y. Zhao, A. Patzer, T. Herdegen, P. Gohlke, and J. Culman, "Activation of cerebral peroxisome proliferator-activated receptors γ promotes neuroprotection by attenuation of neuronal cyclooxygenase-2 overexpression after focal cerebral ischemia in rats," *FASEB Journal*, vol. 20, no. 8, pp. 1162–1175, 2006.

[36] M. T. Heneka, D. L. Feinstein, E. Galea, M. Gleichmann, U. Wüllner, and T. Klockgether, "Peroxisome proliferator-activated receptor γ agonists protect cerebellar granule cells from cytokine-induced apoptotic cell death by inhibition of inducible nitric oxide synthase," *Journal of Neuroimmunology*, vol. 100, no. 1-2, pp. 156–168, 1999.

[37] M. P. Pereira, O. Hurtado, A. Cárdenas, et al., "The nonthiazolidinedione PPARγ agonist L-796,449 is neuroprotective in experimental stroke," *Journal of Neuropathology and Experimental Neurology*, vol. 64, no. 9, pp. 797–805, 2005.

[38] X. Zhao, Y. Zhang, R. Strong, J. C. Grotta, and J. Aronowski, "15d-prostaglandin J_2 activates peroxisome proliferator-activated receptor-γ, promotes expression of catalase, and reduces inflammation, behavioral dysfunction, and neuronal loss after intracerebral hemorrhage in rats," *Journal of Cerebral Blood Flow & Metabolism*, vol. 26, no. 6, pp. 811–820, 2006.

[39] A. Bernardo, G. Levi, and L. Minghetti, "Role of the peroxisome proliferator-activated receptor-γ (PPAR-γ) and its natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in the regulation of microglial functions," *European Journal of Neuroscience*, vol. 12, no. 7, pp. 2215–2223, 2000.

[40] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[41] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[42] T. V. Petrova, K. T. Akama, and L. J. Van Eldik, "Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 8, pp. 4668–4673, 1999.

[43] T. Dehmer, M. T. Heneka, M. Sastre, J. Dichgans, and J. B. Schulz, "Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with $I_\kappa B\alpha$ induction and block of $NF_\kappa B$ and iNOS activation," *Journal of Neurochemistry*, vol. 88, no. 2, pp. 494–501, 2004.

[44] M. Niino, K. Iwabuchi, S. Kikuchi, et al., "Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-γ," *Journal of Neuroimmunology*, vol. 116, no. 1, pp. 40–48, 2001.

[45] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of β- amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists," *Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[46] T. Breidert, J. Callebert, M. T. Heneka, G. Landreth, J. M. Launay, and E. C. Hirsch, "Protective action of the peroxisome proliferator-activated receptor-γ agonist pioglitazone in a mouse model of Parkinson's disease," *Journal of Neurochemistry*, vol. 82, no. 3, pp. 615–624, 2002.

[47] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[48] A. Diab, C. Deng, J. D. Smith, et al., "Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis," *Journal of Immunology*, vol. 168, no. 5, pp. 2508–2515, 2002.

[49] P. G. Popovich, Z. Guan, P. Wei, I. Huitinga, N. van Rooijen, and B. T. Stokes, "Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury," *Experimental Neurology*, vol. 158, no. 2, pp. 351–365, 1999.

[50] J. R. Bethea, H. Nagashima, M. C. Acosta, et al., "Systemically administered interleukin-10 reduces tumor necrosis factor-α production and significantly improves functional recovery following traumatic spinal cord injury in rats," *Journal of Neurotrauma*, vol. 16, no. 10, pp. 851–863, 1999.

[51] D. P. Stirling, K. Khodarahmi, J. Liu, et al., "Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury," *Journal of Neuroscience*, vol. 24, no. 9, pp. 2182–2190, 2004.

[52] D. Gris, D. R. Marsh, M. A. Oatway, et al., "Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function," *Journal of Neuroscience*, vol. 24, no. 16, pp. 4043–4051, 2004.

[53] S. M. Lee, T. Y. Yune, S. J. Kim, et al., "Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat," *Journal of Neurotrauma*, vol. 20, no. 10, pp. 1017–1027, 2003.

[54] J. E. A. Wells, R. J. Hurlbert, M. G. Fehlings, and V. W. Yong, "Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice," *Brain*, vol. 126, no. 7, pp. 1628–1637, 2003.

[55] A. R. Blight, "Delayed demyelination and macrophage invasion: a candidate for secondary cell damage in spinal cord injury," *Central Nervous System Trauma*, vol. 2, no. 4, pp. 299–315, 1985.

[56] R. F. Gledhill, B. M. Harrison, and W. I. McDonald, "Demyelination and remyelination after acute spinal cord compression," *Experimental Neurology*, vol. 38, no. 3, pp. 472–487, 1973.

[57] M. O. Totoiu and H. S. Keirstead, "Spinal cord injury is accompanied by chronic progressive demyelination," *Journal of Comparative Neurology*, vol. 486, no. 4, pp. 373–383, 2005.

[58] S. D. Grossman, L. J. Rosenberg, and J. R. Wrathall, "Temporal—spatial pattern of acute neuronal and glial loss after spinal cord contusion," *Experimental Neurology*, vol. 168, no. 2, pp. 273–282, 2001.

[59] M. J. Crowe, J. C. Bresnahan, S. L. Shuman, J. N. Masters, and M. S. Beattie, "Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys," *Nature Medicine*, vol. 3, no. 1, pp. 73–76, 1997, erratum in *Nature Medicine*, vol. 3, no. 2, p. 240, 1997.

[60] S. Casha, W. R. Yu, and M. G. Fehlings, "Oligodendroglial apoptosis occurs along degenerating axons and is associated with FAS and p75 expression following spinal cord injury in the rat," *Neuroscience*, vol. 103, no. 1, pp. 203–218, 2001.

[61] H. P. Raikwar, G. Muthian, J. Rajasingh, C. Johnson, and J. J. Bright, "PPARγ antagonists exacerbate neural antigen-specific Th1 response and experimental allergic encephalomyelitis," *Journal of Neuroimmunology*, vol. 167, no. 1-2, pp. 99–107, 2005.

[62] C. B. Duvanel, P. Honegger, H. Pershadsingh, D. Feinstein, and J.-M. Matthieu, "Inhibition of glial cell proinflammatory activities by peroxisome proliferator-activated receptor γ agonist confers partial protection during antimyelin oligodendrocyte glycoprotein demyelination in vitro," *Journal of Neuroscience Research*, vol. 71, no. 2, pp. 246–255, 2003.

[63] A. R. Blight and V. Decrescito, "Morphometric analysis of experimental spinal cord injury in the cat: the relation of injury intensity to survival of myelinated axons," *Neuroscience*, vol. 19, no. 1, pp. 321–341, 1986.

[64] T. Shimazu, I. Inoue, N. Araki, et al., "A peroxisome proliferator-activated receptor-γ agonist reduces infarct size in transient but not in permanent ischemia," *Stroke*, vol. 36, no. 2, pp. 353–359, 2005.

[65] M. T. Heneka, M. Sastre, L. Dumitrescu-Ozimek, et al., "Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV717I transgenic mice," *Brain*, vol. 128, no. 6, pp. 1442–1453, 2005.

[66] M. Sastre, I. Dewachter, S. Rossner, et al., "Nonsteroidal anti-inflammatory drugs repress β-secretase gene promoter activity by the activation of PPARγ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 2, pp. 443–448, 2006.

[67] J. J. Bright, C. Natarajan, G. Muthian, Y. Barak, and R. M. Evans, "Peroxisome proliferator-activated receptor-γ-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis," *Journal of Immunology*, vol. 71, no. 11, pp. 5743–5750, 2003.

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Review Article

Therapeutic Potential of PPARγ Activation in Stroke

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Stroke (focal cerebral ischemia) is a leading cause of death and disability among adult population. Many pathological events including inflammation and oxidative stress during the acute period contributes to the secondary neuronal death leading the neurological dysfunction after stroke. Transcriptional regulation of genes that promote these pathophysiological mechanisms can be an effective strategy to minimize the poststroke neuronal death. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors known to be upstream to many inflammatory and antioxidant genes. The goal of this review is to discuss the therapeutic potential and putative mechanisms of neuroprotection following PPAR activation after stroke.

1. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)

PPAR and retinoid X receptor (RXR) are ligand-activated transcription factors of the nuclear hormone receptor superfamily [1, 2]. PPAR exists as 3 isoforms (α, γ, and δ/β) with distinct natural agonists for each isoform. RXR also exists as 3 isoforms (α, β, and γ), but all 3 can be activated by common ligands [3]. Both RXR and PPAR are composed of a ligand binding domain (LBD) and a DNAbinding domain (DBD) [4]. When their respective ligands bind to PPAR and RXR, they form a heterodimeric complex which recruits other coactivators including PPAR coactivator-1 and -2, PPAR-binding protein, PPAR-interacting protein, CREB-binding protein and steroid receptor coactivator-1. This complex binds to the promoter regions of specific genes that contain a regulatory element known as the peroxisome proliferator response element (PPRE; AGGTCA-AGGTCA repeats) which either activates or transrepresses the target genes [1, 5]. Binding of a specific agonist to PPAR is a prerequisite for coactivator binding. In the absence of a ligand, the PPARγ:RXR complex can recruit corepressor complexes and bind to PPRE, suppressing the transcription of target genes [6]. Thus PPARs can control the gene expression positively as well as negatively.

2. FUNCTIONAL SIGNIFICANCE OF PPARs

In the mammalian body, PPARs control glucose and lipid metabolism, cell proliferation and differentiation [1, 7]. In particular, the PPARγ isoform behaves as a "molecular sensor," binding a wide range of molecules involved in metabolism, and has been studied extensively in diabetes and obesity due to its role in regulating glucose metabolism [8, 9]. A class of synthetic, insulin-sensitizing compounds called thiazolidinediones (TZDs) have emerged as potent, exogenous agonists of PPARγ and are being prescribed for type-2 diabetes [1, 10]. PPARγ shows a highly restricted pattern of expression. It is present at a high amount in adipose tissue where it regulates adipocyte differentiation and lipid metabolism [5]. Its expression is also very high in cells of the immune system such as monocytes/macrophages, B and T cells [11]. In the normal adult brain, PPARγ shows a relatively low level of expression primarily limited to the granule cells of the hippocampal dentate gyrus [11]. Some PPARγ expression is also present in the caudate putamen and globus pallidus of the basal ganglia, thalamus, and the piriform cortex [2]. Recent studies indicated that microglia and astrocytes, the cell types that play a significant role in the inflammatory responses of the CNS show high expression levels of PPARγ [12]. More recently, several TZDs including

the United States Food and Drug Administration (FDA) approved rosiglitazone and pioglitazone were shown to control inflammation in peripheral organs as well as CNS [13, 14].

3. PPAR LIGANDS

The endogenous agonists of PPARα include eicosinoids-like leukotriene B_4 and 8(S)-hydroxy-eicosatetraenoic acid [15]. Whereas 15-deoxy-delta-12,14-prostaglandin-J2 (15dPGJ2), and several oxidized metabolites of hydroxyl-eicosatetraenoic acid and hydroxyl-eicosadecaenoic acid are the natural ligands for PPARγ [16]. Many prostanoids are the natural ligands for PPARδ/β [17]. The 3 RXR isoforms use common ligands that include 9-cis retinoic acid, docosahexaenoic acid, and phytanic acid [3].

4. PROMOTERS OF STROKE-INDUCED BRAIN DAMAGE

Following stroke, while the ischemic core undergoes irreversible damage, the penumbra (tissue surrounding the core) can potentially be rescued with timely therapeutic intervention [18]. Typically, the penumbra is much larger in volume than the core to start with, but as the cell death progresses with time, the infarct grows in size engulfing penumbra [19]. The secondary neuronal death that eventually precipitates the long-term neurological dysfunction after stroke is caused by many synergistic pathophysiological mechanisms involving various cell types. In particular, massive inflammation that starts immediately and continues for days after focal ischemia is a major promoter of ischemic neuronal death [20]. In core of injury, anoxic depolarization promotes calcium and potassium release leading to neurotransmitter glutamate release. This follows with a wave of spreading depression which promotes further glutamate release in penumbra. Increased extracellular glutamate promotes excitotoxic secondary neuronal death in core as well as penumbra. Immediately after stroke, due to lack of oxygen and glucose, the ionic gradients across cell membranes collapse leading to water influx and edema in CNS. In addition, mitochondrial failure leads to endoplasmic reticulum (ER) stress and oxidative stress. This is followed by the increased expression of inflammatory genes and infiltration of leukocytes into brain parenchyma. All these pathophysiological events are thought to synergistically promote the postischemic neuronal death [21].

5. INFLAMMATION AFTER STROKE

In a normal brain, the blood-brain barrier (BBB) controls the infiltration of white blood cells into brain parenchyma. However, following ischemia induction of the adhesion molecules like intercellular adhesion molecule-1 (ICAM1), E-selectin, and P-selectin on the endothelial cells promotes leukocyte adherence and extravasation [20]. The infiltrated macrophages and neutrophils activate resident microglia and astrocytes [22]. Following stroke, leukocytes as well as neurons, astrocytes, microglia, and oligodendrocytes generate proinflammatory mediators including cytokines like interleukin (IL)-6 and IL-1β, chemokines like macrophage inflammatory protein-1α and monocyte chemoattractant protein-1 (MCP1), prostaglandins and free radicals which exacerbate postischemic secondary neuronal death [23, 24].

6. ROLE OF TRANSCRIPTION FACTORS IN POSTISCHEMIC INFLAMMATION

Transcription factors play a central role in modulating inflammation by controlling the expression of cytokines, chemokines, and other inflammatory genes. Ischemia is a known stimulator of many transcription factors including hypoxia inducible factor-1 (HIF1), signal transducer and activator of transcription-3 (STAT3), early growth response-1 (Egr1), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), interferon regulatory factor-1 (IRF1), activating transcription factor-3 (ATF3), cAMP response element binding protein (CREB), cAMP response element modulator (CREM), and nuclear factor-kappa B (NF-κB) that are known to be significantly modulate the postischemic inflammatory gene expression [25–28]. While the transcription factors like STAT3, IRF1, C/EBPβ, NF-κB, ATF3, and EGR1 promote neuronal damage by inducing inflammatory genes [26–31], transcription factors like HIF1, Nrf2, PPARα, PPARγ, and CREBare thought to be beneficial as they curtail the expression of genes that promote either inflammation or oxidative stress [32–36]. Drugs that target transcription factors could be effective as they act upstream to gene expression, thus curtailing the inflammation and other destructive pathways.

7. ANTI-INFLAMMATORY EFFECTS OF PPARγ ACTIVATION IN THE PERIPHERAL ORGANS

Many recent studies demonstrated that PPARγ agonists exert significant protection in various animal models of neurological and cardiovascular disorders [37–40]. Activated macrophages release proinflammatory cytokines such as tumor necrosis factor-α (TNFα) and IL6 and free radicals such as nitric oxide (NO) and superoxide. PPARγ activation by its agonists was shown to inhibit the expression of inducible NO synthase (iNOS) and inflammatory cytokine production in macrophages and endothelial cells [5, 41, 42]. PPARγ agonists were also shown to reduce ROSformation in coronary artery endothelial cells and cardiac fibroblasts [43, 44]. Pioglitazone was shown to curtail ICAM1 and MCP1 expression leading to decreased macrophage infiltration after cardiac ischemia leading to curtailed myocardial damage in rats [45]. PPARγ natural ligand 15dPGJ2 was shown to prevent the expression of IL6, IL1β, and TNFα in phorbol 12-myristate 13-acetate-stimulated monocytes [42]. The systemic inflammation in joints of rheumatoid arthritis patients and in the pancreas of diabetics was also shown to be minimized by treatment of PPARγ agonists [8, 46–48]. Another agonist of PPARγ, rosiglitazone treatment was shown to limit neutrophil infiltration, nitrotyrosine formation and lipid peroxidation following experimental pancreatitis in mice [49]. Rosiglitazone was also shown

to decrease matrix metalloproteinase (MMP)-9 expression, T-cell activation, and TNFα and amyloid-A levels in diabetic patients with coronary artery disease, thereby curtailing the inflammatory response [50]. Both pioglitazone and rosiglitazone were shown to decrease inflammation in kidney to prevent nephropathy resulting from diabetes and hypertension [51]. In experimental animals, 15dPGJ2 was shown to inhibit NF-κB activation and other proinflammatory proteins like activating protein-1 (AP-1), iNOS, and ICAM1, thereby decreasing oxidative stress to protect kidneys from ischemic damage [51–53]. Upon activation, TZD pretreated peripheral blood monocytes show decreased cytokine release and altered inflammatory gene expression [42, 54]. It was shown that PPARγ activation antagonizes transcription factors STAT, NF-κB, and AP1 by decreasing their DNAbinding leading to decreased expression of the downstream genes iNOS, MMP9, and scavenger receptor-A [41, 55, 56].

8. PPARγ ACTIVATION AND STROKE

Massive inflammation is a known precipitator of stroke-induced brain damage [20, 21, 57]. Many anti-inflammatory compounds including minocycline, curcumin, caffeic acid phenyl ester and Brazilein can limit cerebral inflammation, and thus ischemic neuronal death [58–61]. As brain damage following focal ischemia is known to be mediated by many synergistic mechanisms including edema, ionic imbalance, apoptosis, oxidative stress, and ER stress, combination of drugs that prevent some if not all these pathophysiological changes might be needed to efficiently control ischemic neuronal death.

Consistent with the known benefits of PPARγ activation in conditions of inflammation, several animal studies have demonstrated the therapeutic potential of TZDs in improving postischemic functional outcome. As 2 TZDs rosiglitazone and pioglitazone are currently approved by FDA for type-2 diabetes treatment and as the incidence of stroke and stroke-induced brain damage are higher in type-2 diabetics, these studies assume great importance [32, 62, 63]. Following transient focal ischemia in rodents, cerebral PPARγ expression significantly elevates, especially in the peri-infarct area, and treatment with PPARγ agonists transrepress the expression of many downstream proinflammatory genes [64]. Furthermore, rosiglitazone or pioglitazone treatment increases PPARγ translocation to the nucleus in neurons that will be enhanced by RXR agonist retinoic acid [64, 65].

Both pretreatment as well as posttreatment with TZDs was shown to induce neuroprotection after focal ischemia [32, 66–69]. In addition, TZD treatment was observed to be effective irrespective of the route of administration. Postischemic neuroprotection was observed following intraperitoneal (i.p.) injections, intracerebroventricular (i.c.v.) infusion as well as feeding animals with chow enriched with a TZD [32, 66–68]. In adult rats and mice, pretreatment with troglitazone, rosiglitazone, or pioglitazone (i.p.) prior to ischemia was shown to decrease the infarct volume, microglial activation, macrophage infiltration, and expression of proinflammatory genes cyclooxygenase-2 (COX2), iNOS, and IL-1β mRNA in the ischemic hemisphere [32, 66, 68]. The rosiglitazone neuroprotection was observed to be completely reversed by treating rats with GW9662 (a specific PPARγ antagonist) indicating a direct involvement of PPARγ [32]. Pioglitazone when infused i.c.v. for 5 days prior to and 2 days after focal ischemia significantly increased the sensory neurological scores and reduced edema and infarct volume [67]. In another study, rats tube fed with rosiglitazone for 7 days prior to and 3 days after transient focal ischemia showed increased endothelial NO synthase (eNOS) expression and neoangiogenesis leading to ischemic tolerance [70]. Pioglitazone pretreatment was not observed to be neuroprotective following permanent focal ischemia in rats, suggesting that the beneficial effects of TZDs are limited to reperfusion-induced damage [71]. Importantly, TZD-induced neuroprotection was also observed in hypertensive and diabetic rodents subjected to transient focal ischemia [32].

9. PPAR ACTS TOGETHER WITH OTHER TRANSCRIPTION FACTORS

The transrepression of inflammatory genes by PPAR acts in cooperation with many other transcription factors including NF-κB, AP-1, Egr1, and c/EBPβ, and by inhibiting the ubiquitylation/degradation of corepressor proteins via sumoylation [72]. Vascular inflammation was shown to be controlled by the interaction of PPARγ and c/EBPβ by negatively regulating the expression of inflammatory genes like IL-6, IL1β, and TNFα [73]. This is made possible by the presence of tandem repeats of c/EBPβ motif in the PPARγ promoter region enabling transactivation of PPARγ gene. The PPAR:RXR heterodimer complex also competes with the coactivator complexes as well as interacts directly with other transcription factors to regulate their function. In addition, TZDs can have PPARγ-independent actions that include mitochondrial dysfunction-related, stress-response, increased astrocyte, glucose uptake and lactate production, and modulation of the mitochondrial protein MitoNeet [74, 75].

10. EFFICACY OF PPARγ AGONISTS AFTER FOCAL ISCHEMIA

Of the two FDA-approved TZDs, pioglitazone is known to cross BBB more efficiently than rosiglitazone, but the affinity of pioglitazone to PPARγ is 10 times lower (Kd of ~400 nM) than for rosiglitazone (Kd of ~40 nM) [1, 76]. In addition to stimulating PPARγ, pioglitazone also functions as a partial agonist of PPARα, whereas rosiglitazone functions as a pure PPARγ agonist [13]. To make things complicated, recent studies demonstrated that to induce the same degree of neuroprotection following focal ischemia or SCI, comparable doses of pioglitazone and rosiglitazone are needed [32, 77].

11. NONINFLAMMATION-RELATED NEUROPROTECTIVE ACTIONS OF TZDs

Although preventing inflammation seems to be the major neuroprotective mechanism of PPAR agonists after stroke,

both PPARγ and PPARα agonists were shown to induce other beneficial effects like reducing oxidative stress, increasing endothelial relaxation, and preventing apoptosis in the postischemic brain [63, 78, 79]. When oxidative stress was induced in immortalized mouse hippocampal cells by exposing to glutamate or hydrogen peroxide, PPARγ agonists protected the cells from death [39]. Transient focal ischemia is known to promote reactive oxygen species (ROS) production and reduce glutathione levels (which scavenge ROS) simultaneously [80]. This leads to enormous oxidative stress and neuronal death. The cytosolic antioxidant enzyme, endothelial copper/zinc-superoxide dismutase (Cu/Zn-SOD) is known to decrease oxygen-free radicals to mitigate eNOS inactivation [81]. Catalase, the other major antioxidant enzyme which is very active in peroxisomes of both neurons and glial cells [82, 83] protects cells by quickly degrading hydrogen peroxide. Neurons are extremely labile to oxidative damage and cellular stress is a known inducer of both Cu/Zn-SOD and catalase expression to counter the oxidative stress and to protect neurons following focal ischemia. As the promoters of SOD and catalase genes contain PPRE, they are directly upregulated when PPARγ is activated [81, 84].

Recent studies from our laboratory showed that in normotensive and hypertensive rodents subjected to transient focal ischemia, rosiglitazone treatment significantly increases Cu/Zn-SOD and catalase activity in the peri-infarct region which might be responsible for the observed neuroprotection [32]. In addition, rosiglitazone also decreased COX-2 and iNOS levels (indicating reduced production of ROS and NO) in peri-infarct neurons [34]. It was also reported that both pioglitazone and rosiglitazone significantly prevent glutathione depletion following focal ischemia in adult rats [80].

As ROS promotes apoptosis and TZDs minimize ROS formation, PPARγ activation was thought to prevent postischemic apoptotic neuronal death [85, 86]. A recent study showed that rosiglitazone treatment decreases caspase-3 levels leading to reduced apoptotic cell death following focal ischemia [87]. This seems to be a direct PPARγ downstream effect as pretreating animals with the PPARγ antagonist GW9662 prevented the antiapoptotic actions of rosiglitazone [87]. Pioglitazone treatment was also shown to promote the expression of antiapoptotic gene Bcl2 while simultaneously preventing the expression of proapoptotic gene Bax in the peri-infarct regions of brain following focal ischemia [13, 88].

12. PPARγ AGONIST-INDUCED NEUROPROTECTION IN HUMAN STROKE SUBJECTS

A recent clinical trial, named the Prospective Pioglitazone Clinical Trialin Macrovascular events (proactive) started evaluating if pioglitazone treatment can prevent the macrovascularevents in type-2 diabetes [89, 90]. This extensive study evaluated 5, 238 patients in 19 countries. In particular, one prespecified subgroup analysis evaluated the effect of pioglitazone in patients with (n = 984) or without (n = 2867) a history of stroke and observed a 16% relative risk reduction in the pioglitazone group compared to placebo group [91]. In addition, within the group of patients with a previous stroke, pioglitazone therapy decreased the risk of recurrent stroke by 47% compared to placebo over 3 years. But pioglitazone had no effect on decreasing first strokes over this period. While this shows an encouraging trend for stroke patients, serious heart failure was observed to be increased significantly in the pioglitazone group compared to placebo group. The complete details and results of the proactive trial can be viewed at the website http://www.proactive-results.com/index.htm. Yki-Järvinen [92] commented critically on this trial that pioglitazone group showed increased edema and increased incidence of pneumonia. Furthermore, the weight gain was 4 kg greater in the pioglitazone over placebo group which is undesirable. A recent study also showed that pioglitazone or rosiglitazone therapy significantly enhanced the functional recovery in a group of 30 type-2 diabetes patients admitted in the hospital for acute stroke rehabilitation [93].

13. BENEFICIAL EFFECT OF PPARγ ACTIVATION IN OTHER CNS INJURIES

PPARγ activation by TZDs was shown to prevent inflammation and neuronal death in several in vitro and in vivo models of CNS diseases [40, 94–97]. In patients suffering with Alzheimer disease, PPARγ activation was shown to prevent TNFα and iNOS expression in macrophages, thus limiting the inflammation and cognitive impairment [40, 98]. TZDs were known to significantly reduce the dopaminergic neuronal loss leading to improved neurological status in Parkinson's disease [99]. Using the rodent model of multiple sclerosis (experimental autoimmune encephalomyelitis), PPARγ agonist treatment was shown to suppress activation of T-cells, microglia, and macrophages thus decreasing proinflammatory factor formation leading to improved neurological outcome [94, 96, 100]. Pioglitazone oral treatment was reported to decrease the microglial activation, motor neuron loss, and muscular atrophy in transgenic mice overexpressing SOD1-G93A (an animal model of Amyotrophic lateral sclerosis) with pioglitazone [101, 102]. These mice also showed increased anti-inflammatory gene expression upon treatment with pioglitazone [101]. More recently, two studies showed the beneficial effects of treating rodents with PPARγ agonists following spinal cord injury (SCI) [77, 103]. Our laboratory demonstrated that both rosiglitazone and pioglitazone decreases inflammatory cell activation and inflammatory gene expression leading to smaller lesion size, better motor recovery, and less neuropathic pain after SCI in rats [77]. Importantly, we showed that pretreating rats with the PPARγ antagonist GW9662 prevents many beneficial effects of TZDs following SCI indicating a direct mediation of PPARγ in promoting post-SCI neuronal recovery [77].

14. PPARγ-INDEPENDENT NEUROPROTECTIVE ACTIONS OF PPARγ AGONISTS

While the anti-inflammatory and neuroprotective actions of PPARγ agonists are expected to be mediated via PPARγ

stimulation, some studies suggested that PPARγ agonists also induce many beneficial effects via PPARγ-independent mechanisms as well. 15dPGJ2 was shown to prevent astroglial and microglial activation by bacterial endotoxins without involving PPARγ [12]. The anti-inflammatory action of 15dPGJ2 was shown to be mediated by binding to and inactivating inhibitor of kappa B (IκB) kinase and by alkylating the p50/p65 dimers and thus preventing the activation of NF-κB without involving PPARγ [52, 55]. Following focal ischemia, the proinflammatory actions of the cytokine IL6 are known to be mediated by the activation of IL6 receptor-associated Janus kinases (JAKs) and their downstream STAT family of transcription factors [25]. Suppressor of cytokine signalling (SOCS) proteins act as negative feedback regulators and inhibit JAK and STAT phosphorylation, thus preventing the upregulation and binding of cytokines to their receptors after an acute CNS insult [32, 104]. Recent studies from our group and others demonstrated that rosiglitazone and 15dPGJ2 induce SOCS3 expression and prevent JAK2 and STAT3 phosphorylation [32, 105]. Thus the neuroprotective actions of PPARγ agonists might also mediated by direct actions on JAK-STAT-SOCS pathway. Our recent studies also showed that pioglitazone treatment after SCI induces the heat shock protein (HSP)-27, HSP70, and HSP32 (hemeoxygenase-1) which induce neuroprotection [104].

15. ACTIVATION OF OTHER PPAR ISOFORMS ALSO INDUCES NEUROPROTECTION

In addition to PPARγ, PPARα, and PPARβ/δ, activation was also shown to significantly prevent inflammation and induce protection after injury to CNS as well as peripheral organs. PPARα activation is known to induce neuroprotection after focal ischemia [36, 78]. PPARα plays a very important regulatory role in response to injury or stress. As PPARα is known to be expressed when monocytes differentiate into macrophages, it influences postinjury inflammatory reactions [105]. Furthermore, agonist-induced activation of PPARα increases IκBα levels leading to an inhibition of NF-κB [106]. PPARα activation decreases the level of proatherosclerotic fibrinogen and C-reactive protein in experimental animals [107]. Fenofibrate, a potent exogenous agonist of PPARα, inhibits left ventricular hypertrophy by stimulating free fatty acid uptake and β-oxidation [63]. Fenofibrate pretreatment reduces the susceptibility of mice deficient in apolipoprotein-E, and decreases the infarct volume in wild type mice subjected to focal cerebral ischemia [78]. Poststroke neuroprotection induced by PPARα agonists will be mediated by both cerebral and vascular mechanisms. Fenofibrate treatment is known to decrease vascular endothelial dysfunction and improves endothelium-dependent vasodilatation in patients with hypertriglyceridemia [91]. Recent studies showed that a PPARα/γ dual agonist bezafibrate decreases anaerobic metabolism and there by prevents death in gerbils subjected to global cerebral ischemia [108]. Fibrates are also reported to prevent secondary neuronal death by oxidative stress by enhancing the expression of antioxidant enzyme, Cu/Zn-SOD, and by decreasing vascular cell adhesion molecule-1 expression in CNS blood vessels, possibly by inhibiting the NF-κB pathway [98, 109]. The functional significance of PPARβ/δ in preventing CNS inflammation is not studied in detail. However, the PPARβ/δ agonists L-165041, and GW501516 were shown to significantly decrease focal ischemia-induced infarction and brain damage in adult rats [110]. In rodents, PPARβ/δ agonists were also demonstrated to prevent striatal dopamine loss after 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine administration [110]. PPARβ/δ agonist GW0742 was reported to inhibit lipopolysaccharide-induced TNFα secretion in cardiomyocytes [111].

16. CONCLUSIONS

Despite decades of research, no therapies that can prevent the secondary neuronal death and the ensuing neurological deficits after stroke are currently available. Many pathological mechanisms including inflammation, ionic imbalance, excitotoxicity, edema, oxidative stress, and ER stress synergistically promote the poststroke secondary neuronal death. Hence therapeutics that simultaneously targets several of these mechanisms with minimal side effects is extremely useful in stroke therapy. PPARγ agonists like rosiglitazone and pioglitazone are FDA approved and being prescribed to millions of type-2 diabetics allover the world. The benefit of these compounds seems to be their potential to influence multiple molecular mechanisms. For example, they are known to minimize the harmful events like inflammation and oxidative stress at the same time promote the antioxidant defence and protein chaperones. Hence PPARγ agonists might be an important class of drugs for use in stroke therapy. The benefits of PPARγ agonist treatment was also observed in other acute CNS injuries like SCIas well as chronic neurodegenerative disorders like multiple sclerosis, Alzheimer's disease, and Parkinson's disease increasing the promise of these compounds as future neuroprotective therapies.

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REFERENCES

[1] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[2] S. Moreno, S. Farioli-Vecchioli, and M. P. Cerù, "Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS," *Neuroscience*, vol. 123, no. 1, pp. 131–145, 2004.

[3] A. R. de Lera, W. Bourguet, L. Altucci, and H. Gronemeyer, "Design of selective nuclear receptor modulators: RAR and RXR as a case study," *Nature Reviews Drug Discovery*, vol. 6, no. 10, pp. 811–820, 2007.

[4] D. J. Mangelsdorf, C. Thummel, M. Beato, et al., "The nuclear receptor superfamily: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[5] R. Cunard, M. Ricote, D. DiCampli, et al., "Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors," *Journal of Immunology*, vol. 168, no. 6, pp. 2795–2802, 2002.

[6] C. A. Argmann, T.-A. Cock, and J. Auwerx, "Peroxisome proliferator-activated receptor γ: the more the merrier?" *European Journal of Clinical Investigation*, vol. 35, no. 2, pp. 82–92, 2005.

[7] K. Schoonjans, B. Staels, and J. Auwerx, "The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation," *Biochimica et Biophysica Acta*, vol. 1302, no. 2, pp. 93–109, 1996.

[8] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[9] B. Desvergne, L. Michalik, and W. Wahli, "Be fit or be sick: peroxisome proliferator-activated receptors are down the road," *Molecular Endocrinology*, vol. 18, no. 6, pp. 1321–1332, 2004.

[10] L. C. Bell-Parikh, T. Ide, J. A. Lawson, P. McNamara, M. Reilly, and G. A. FitzGerald, "Biosynthesis of 15-deoxy-$\Delta^{12,14}$-PGJ_2 and the ligation of PPARγ," *Journal of Clinical Investigation*, vol. 112, no. 6, pp. 945–955, 2003.

[11] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): Tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[12] A. Bernardo, G. Levi, and L. Minghetti, "Role of the peroxisome proliferator-activated receptor-γ (PPAR-γ) and its natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in the regulation of microglial functions," *European Journal of Neuroscience*, vol. 12, no. 7, pp. 2215–2223, 2000.

[13] J. Sakamoto, H. Kimura, S. Moriyama, et al., "Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone," *Biochemical and Biophysical Research Communications*, vol. 278, no. 3, pp. 704–711, 2000.

[14] D. J. Kudzma, "Effects of thiazolidinediones for early treatment of type 2 diabetes mellitus," *American Journal of Managed Care*, vol. 8, supplement 16, pp. S472–S482, 2002.

[15] A. A. Bogan, F. E. Cohen, and T. S. Scanlan, "Natural ligands of nuclear receptors have conserved volumes," *Nature Structural Biology*, vol. 5, no. 8, pp. 679–681, 1998.

[16] T. Shiraki, N. Kamiya, S. Shiki, T. S. Kodama, A. Kakizuka, and H. Jingami, "α,β-unsaturated ketone is a core moiety of natural ligands for covalent binding to peroxisome proliferator-activated receptor γ," *Journal of Biological Chemistry*, vol. 280, no. 14, pp. 14145–14153, 2005.

[17] F. Chang, L. A. Jaber, H. D. Berlie, and M. B. O'Connell, "Evolution of peroxisome proliferator-activated receptor agonists," *Annals of Pharmacotherapy*, vol. 41, no. 6, pp. 973–983, 2007.

[18] S. T. Olsen, P. Bruhn, and G. Oberg, "An ischemic penumbra as the cause of neurologic deficit in humans," *Journal of Cerebral Blood Flow & Metabolism*, pp. S7–S8, 1985.

[19] P. J. Camarata, R. C. Heros, and R. E. Latchaw, ""Brain attack": the rationale for treating stroke as a medical emergency," *Neurosurgery*, vol. 34, no. 1, pp. 144–158, 1994.

[20] S. M. Lucas, N. J. Rothwell, and R. M. Gibson, "The role of inflammation in CNS disease and injury," *British Journal of Pharmacology*, vol. 147, no. S1, pp. S232–S240, 2006.

[21] P. Lipton, "Ischemic cell death in brain neurons," *Physiological Reviews*, vol. 79, no. 4, pp. 1431–1568, 1999.

[22] U. Dirnagl, C. Iadecola, and M. A. Moskowitz, "Pathobiology of ischaemic stroke: an integrated view," *Trends in Neurosciences*, vol. 22, no. 9, pp. 391–397, 1999.

[23] D. Stanimirovic and K. Satoh, "Inflammatory mediators of cerebral endothelium: a role in ischemic brain inflammation," *Brain Pathology*, vol. 10, no. 1, pp. 113–126, 2000.

[24] C. Iadecola and M. Alexander, "Cerebral ischemia and inflammation," *Current Opinion in Neurology*, vol. 14, no. 1, pp. 89–94, 2001.

[25] I. Satriotomo, K. K. Bowen, and R. Vemuganti, "JAK2 and STAT3 activation contributes to neuronal damage following transient focal cerebral ischemia," *Journal of Neurochemistry*, vol. 98, no. 5, pp. 1353–1368, 2006.

[26] V. L. Raghavendra Rao, K. K. Bowen, V. K. Dhodda, et al., "Gene expression analysis of spontaneously hypertensive rat cerebral cortex following transient focal cerebral ischemia," *Journal of Neurochemistry*, vol. 83, no. 5, pp. 1072–1086, 2002.

[27] A. M. Planas, M. A. Soriano, M. Berruezo, et al., "Induction of Stat3, a signal transducer and transcription factor, in reactive microglia following transient focal cerebral ischaemia," *European Journal of Neuroscience*, vol. 8, no. 12, pp. 2612–2618, 1996.

[28] A. Lu, Y. Tang, R. Ran, J. F. Clark, B. J. Aronow, and F. R. Sharp, "Genomics of the periinfarction cortex after focal cerebral ischemia," *Journal of Cerebral Blood Flow and Metabolism*, vol. 23, no. 7, pp. 786–810, 2003.

[29] C. Iadecola, C. A. Salkowski, F. Zhang, et al., "The transcription factor interferon regulatory factor 1 is expressed after cerebral ischemia and contributes to ischemic brain injury," *Journal of Experimental Medicine*, vol. 189, no. 4, pp. 719–727, 1999.

[30] R. Kapadia, K. Tureyen, K. K. Bowen, H. Kalluri, P. F. Johnson, and R. Vemuganti, "Decreased brain damage and curtailed inflammation in transcription factor CCAAT/enhancer binding protein β knockout mice following transient focal cerebral ischemia," *Journal of Neurochemistry*, vol. 98, no. 6, pp. 1718–1731, 2006.

[31] G. Z. Feurstein, X. K. Wang, and G. C. Barone, "Inflammatory mediators of ischemic injury: cytokine gene regulation in stroke," in *Cerebrovascular Disease: Pathophysiology, Diagnosis and Management*, M. D. Ginsberg and J. Bogousslavsky, Eds., pp. 507–531, Blackwell, Malden, Mass, USA, 1998.

[32] K. Tureyen, R. Kapadia, K. K. Bowen, et al., "Peroxisome proliferator-activated receptor-γ agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents," *Journal of Neurochemistry*, vol. 101, no. 1, pp. 41–56, 2007.

[33] A. Y. Shih, P. Li, and T. H. Murphy, "A small-molecule-inducible Nrf2-mediated antioxidant response provides effective prophylaxis against cerebral ischemia in vivo," *Journal of Neuroscience*, vol. 25, no. 44, pp. 10321–10335, 2005.

[34] K. Tanaka, S. Nogawa, D. Ito, et al., "Activated phosphorylation of cyclic AMP response element binding protein is associated with preservation of striatal neurons after focal cerebral ischemia in the rat," *Neuroscience*, vol. 100, no. 2, pp. 345–354, 2000.

[35] M. Bernaudin, A.-S. Nedelec, D. Divoux, E. T. MacKenzie, E. Petit, and P. Schumann-Bard, "Normobaric hypoxia induces

tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain," *Journal of Cerebral Blood Flow and Metabolism*, vol. 22, no. 4, pp. 393–403, 2002.

[36] M. Collino, M. Aragno, R. Mastrocola, et al., "Oxidative stress and inflammatory response evoked by transient cerebral ischemia/reperfusion: effects of the PPAR-α agonist WY14643," *Free Radical Biology and Medicine*, vol. 41, no. 4, pp. 579–589, 2006.

[37] N. S. Wayman, Y. Hattori, M. C. Mcdonald, et al., "Ligands of the peroxisome proliferator-activated receptors (PPAR-γ and PPAR-α) reduce myocardial infarct size," *The FASEB Journal*, vol. 16, no. 9, pp. 1027–1040, 2002.

[38] H.-R. Liu, L. Tao, E. Gao, et al., "Anti-apoptotic effects of rosiglitazone in hypercholesterolemic rabbits subjected to myocardial ischemia and reperfusion," *Cardiovascular Research*, vol. 62, no. 1, pp. 135–144, 2004.

[39] P. Aoun, D. G. Watson, and J. W. Simpkins, "Neuroprotective effects of PPARγ agonists against oxidative insults in HT-22 cells," *European Journal of Pharmacology*, vol. 472, no. 1-2, pp. 65–71, 2003.

[40] G. E. Landreth and M. T. Heneka, "Anti-inflammatory actions of peroxisome proliferator-activated receptor γ agonists in Alzheimer's disease," *Neurobiology of Aging*, vol. 22, no. 6, pp. 937–944, 2001.

[41] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[42] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[43] J. L. Mehta, B. Hu, J. Chen, and D. Li, "Pioglitazone inhibits LOX-1 expression in human coronary artery endothelial cells by reducing intracellular superoxide radical generation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 12, pp. 2203–2208, 2003.

[44] K. Chen, D. Li, X. Zhang, P. L. Hermonat, and J. L. Mehta, "Anoxia-reoxygenation stimulates collagen type-1 and MMP-1 expression in cardiac fibroblasts: modulation by the PPAR-γ ligand pioglitazone," *Journal of Cardiovascular Pharmacology*, vol. 44, no. 6, pp. 682–687, 2004.

[45] H. Ito, A. Nakano, M. Kinoshita, and A. Matsumori, "Pioglitazone, a peroxisome proliferator-activated receptor-γ agonist, attenuates myocardial ischemia/reperfusion injury in a rat model," *Laboratory Investigation*, vol. 83, no. 12, pp. 1715–1721, 2003.

[46] W.-W. Yan, Z.-G. Zhou, Y.-D. Chen, and H.-K. Gao, "Role of COX-2 in microcirculatory disturbance in experimental pancreatitis," *World Journal of Gastroenterology*, vol. 10, no. 14, pp. 2095–2098, 2004.

[47] L. Michalik and W. Wahli, "Involvement of PPAR nuclear receptors in tissue injury and wound repair," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 598–606, 2006.

[48] N. Sattar, D. W. McCarey, H. Capell, and I. B. McInnes, "Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis," *Circulation*, vol. 108, no. 24, pp. 2957–2963, 2003.

[49] S. Cuzzocrea, B. Pisano, L. Dugo, et al., "Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-γ, reduces acute pancreatitis induced by cerulein," *Intensive Care Medicine*, vol. 30, no. 5, pp. 951–956, 2004.

[50] N. Marx, J. Froehlich, L. Siam, et al., "Antidiabetic PPARγ-activator rosiglitazone reduces MMP-9 serum levels in type 2 diabetic patients with coronary artery disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 2, pp. 283–288, 2003.

[51] S. Cuzzocrea, "Peroxisome proliferator-activated receptors γ ligands and ischemia and reperfusion injury," *Vascular Pharmacology*, vol. 41, no. 6, pp. 187–195, 2004.

[52] A. Rossi, P. Kapahi, G. Natoli, et al., "Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase," *Nature*, vol. 403, no. 6765, pp. 103–108, 2000.

[53] A. Sivarajah, P. K. Chatterjee, N. S. A. Patel, et al., "Agonists of peroxisome-proliferator activated receptor-γ reduce renal ischemia/reperfusion injury," *American Journal of Nephrology*, vol. 23, no. 4, pp. 267–276, 2003.

[54] S. L. Tilley, T. M. Coffman, and B. H. Koller, "Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes," *Journal of Clinical Investigation*, vol. 108, no. 1, pp. 15–23, 2001.

[55] D. S. Straus, G. Pascual, M. Li, et al., "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 9, pp. 4844–4849, 2000.

[56] P. Delerive, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors in inflammation control," *Journal of Endocrinology*, vol. 169, no. 3, pp. 453–459, 2001.

[57] M. D. Linnik, R. H. Zobrist, M. D. Hatfield, and W. J. Koroshetz, "Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats," *Stroke*, vol. 24, no. 12, pp. 2002–2009, 1993.

[58] J. Yrjänheikki, T. Tikka, R. Keinänen, G. Goldsteins, P. H. Chan, and J. Koistinaho, "A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 23, pp. 13496–13500, 1999.

[59] J. Jiang, W. Wang, Y. J. Sun, M. Hu, F. Li, and D. Y. Zhu, "Neuroprotective effect of curcumin on focal cerebral ischemic rats by preventing blood-brain barrier damage," *European Journal of Pharmacology*, vol. 561, no. 1–3, pp. 54–62, 2007.

[60] M. Khan, C. Elango, M. A. Ansari, I. Singh, and A. K. Singh, "Caffeic acid phenethyl ester reduces neurovascular inflammation and protects rat brain following transient focal cerebral ischemia," *Journal of Neurochemistry*, vol. 102, no. 2, pp. 365–377, 2007.

[61] J. Shen, H. Zhang, H. Lin, H. Su, D. Xing, and L. Du, "Brazilein protects the brain against focal cerebral ischemia reperfusion injury correlating to inflammatory response suppression," *European Journal of Pharmacology*, vol. 558, no. 1–3, pp. 88–95, 2007.

[62] N. Kagansky, S. Levy, and H. Knobler, "The role of hyperglycemia in acute stroke," *Archives of Neurology*, vol. 58, no. 8, pp. 1209–1212, 2001.

[63] T. Ogata, T. Miyauchi, S. Sakai, Y. Irukayama-Tomobe, K. Goto, and I. Yamaguchi, "Stimulation of peroxisome-proliferator-activated receptor α (PPARα) attenuates cardiac fibrosis and endothelin-1 production in pressure-overloaded rat hearts," *Clinical Science*, vol. 103, supplement 48, pp. 284S–288S, 2002.

[64] N. A. Victor, E. W. Wanderi, J. Gamboa, et al., "Altered PPARγ expression and activation after transient focal

ischemia in rats," *European Journal of Neuroscience*, vol. 24, no. 6, pp. 1653–1663, 2006.

[65] E. Y. Park, I. J. Cho, and S. G. Kim, "Transactivation of the PPAR-responsive enhancer module in chemopreventive glutathione S-transferase gene by the peroxisome proliferator-activated receptor-γ and retinoid X receptor heterodimer," *Cancer Research*, vol. 64, no. 10, pp. 3701–3713, 2004.

[66] S. Sundararajan, J. L. Gamboa, N. A. Victor, E. W. Wanderi, W. D. Lust, and G. E. Landreth, "Peroxisome proliferator-activated receptor-γ ligands reduce inflammation and infarction size in transient focal ischemia," *Neuroscience*, vol. 130, no. 3, pp. 685–696, 2005.

[67] Y. Zhao, A. Patzer, P. Gohlke, T. Herdegen, and J. Culman, "The intracerebral application of the PPARγ-ligand pioglitazone confers neuroprotection against focal ischaemia in the rat brain," *European Journal of Neuroscience*, vol. 22, no. 1, pp. 278–282, 2005.

[68] Y. Luo, W. Yin, A. P. Signore, et al., "Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-γ agonist rosiglitazone," *Journal of Neurochemistry*, vol. 97, no. 2, pp. 435–448, 2006.

[69] M. Allahtavakoli, A. Shabanzadeh, A. Roohbakhsh, and A. Pourshanazari, "Combination therapy of rosiglitazone, a peroxisome proliferator-activated receptor-γ ligand, and NMDA receptor antagonist (MK-801) on experimental embolic stroke in rats," *Basic and Clinical Pharmacology and Toxicology*, vol. 101, no. 5, pp. 309–314, 2007.

[70] K. Chu, S.-T. Lee, J.-S. Koo, et al., "Peroxisome proliferator-activated receptor-γ-agonist, rosiglitazone, promotes angiogenesis after focal cerebral ischemia," *Brain Research*, vol. 1093, no. 1, pp. 208–218, 2006.

[71] T. Shimazu, I. Inoue, N. Araki, et al., "A peroxisome proliferator-activated receptor-γ agonist reduces infarct size in transient but not in permanent ischemia," *Stroke*, vol. 36, no. 2, pp. 353–359, 2005.

[72] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[73] Y. Takata, Y. Kitami, Z.-H. Yang, M. Nakamura, T. Okura, and K. Hiwada, "Vascular inflammation is negatively autoregulated by interaction between CCAAT/enhancer-binding protein-δ and peroxisome proliferator-activated receptor-γ," *Circulation Research*, vol. 91, no. 5, pp. 427–433, 2002.

[74] D. L. Feinstein, A. Spagnolo, C. Akar, et al., "Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key?" *Biochemical Pharmacology*, vol. 70, no. 2, pp. 177–188, 2005.

[75] C. Dello Russo, V. Gavrilyuk, G. Weinberg, et al., "Peroxisome proliferator-activated receptor γ thiazolidinedione agonists increase glucose metabolism in astrocytes," *Journal of Biological Chemistry*, vol. 278, no. 8, pp. 5828–5836, 2003.

[76] P. W. Young, D. R. Buckle, B. C. C. Cantello, et al., "Identification of high-affinity binding sites for the insulin sensitizer rosiglitazone (BRL-49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator-activated receptor γ," *Journal of Pharmacology and Experimental Therapeutics*, vol. 284, no. 2, pp. 751–759, 1998.

[77] S.-W. Park, J.-H. Yi, G. Miranpuri, et al., "Thiazolidinedione class of peroxisome proliferator-activated receptor γ agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1002–1012, 2007.

[78] D. Deplanque, P. Gelé, O. Pétrault, et al., "Peroxisome proliferator-activated receptor-α activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment," *Journal of Neuroscience*, vol. 23, no. 15, pp. 6264–6271, 2003.

[79] R. Bordet, T. Ouk, O. Petrault, et al., "PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases," *Biochemical Society Transactions*, vol. 34, no. 6, pp. 1341–1346, 2006.

[80] M. Collino, M. Aragno, R. Mastrocola, et al., "Modulation of the oxidative stress and inflammatory response by PPAR-γ agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion," *European Journal of Pharmacology*, vol. 530, no. 1-2, pp. 70–80, 2006.

[81] J. Hwang, D. J. Kleinhenz, B. Lassègue, K. K. Griendling, S. Dikalov, and C. M. Hart, "Peroxisome proliferator-activated receptor-γ ligands regulate endothelial membrane superoxide production," *American Journal of Physiology*, vol. 288, no. 4, pp. C899–C905, 2005.

[82] E. Geremia, D. Baratta, S. Zafarana, et al., "Antioxidant enzymatic systems in neuronal and glial cell-enriched fractions of rat brain during aging," *Neurochemical Research*, vol. 15, no. 7, pp. 719–723, 1990.

[83] S. Moreno, E. Mugnaini, and M. P. Ceru, "Immunocytochemical localization of catalase in the central nervous system of the rat," *Journal of Histochemistry and Cytochemistry*, vol. 43, no. 12, pp. 1253–1267, 1995.

[84] G. D. Girnun, F. E. Domann, S. A. Moore, and M. E. C. Robbins, "Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter," *Molecular Endocrinology*, vol. 16, no. 12, pp. 2793–2801, 2002.

[85] B. Herrera, A. M. Álvarez, A. Sánchez, et al., "Reactive oxygen species (ROS) mediates the mitochondrial-dependent apoptosis induced by transforming growth factor β in fetal hepatocytes," *The FASEB Journal*, vol. 15, no. 3, pp. 741–751, 2001.

[86] M. A. Perez-Pinzon, K. R. Dave, and A. P. Raval, "Role of reactive oxygen species and protein kinase C in ischemic tolerance in the brain," *Antioxidants and Redox Signaling*, vol. 7, no. 9-10, pp. 1150–1157, 2005.

[87] T.-N. Lin, W.-M. Cheung, J.-S. Wu, et al., "15d-prostaglandin J_2 protects brain from ischemia-reperfusion injury," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 3, pp. 481–487, 2006.

[88] D. Sulejczak, J. Czarkowska-Bauch, M. Macias, and M. Skup, "Bcl-2 and Bax proteins are increased in neocortical but not in thalamic apoptosis following devascularizing lesion of the cerebral cortex in the rat: an immunohistochemical study," *Brain Research*, vol. 1006, no. 2, pp. 133–149, 2004.

[89] B. Charbonnel, J. Dormandy, E. Erdmann, M. Massi-Benedetti, and A. Skene, "The prospective pioglitazone clinical trial in macrovascular events (PROactive): can pioglitazone reduce cardiovascular events in diabetes? Study design and baseline characteristics of 5,238 patients," *Diabetes Care*, vol. 27, no. 7, pp. 1647–1653, 2004.

[90] J. A. Dormandy, B. Charbonnel, D. J. Eckland, et al., "Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive study (PROspective pioglitazone clinical trial in macrovascular events): a randomised controlled trial," *Lancet*, vol. 366, no. 9493, pp. 1279–1289, 2005.

[91] Y. Huang, G. Dai, Z. Feng, et al., "Effect of micronized fenofibrate on vascular endothelial function in patients with hypertriglyceridemia," *Chinese Medical Journal*, vol. 116, no. 11, pp. 1767–1769, 2003.

[92] H. Yki-Järvinen, "The PROactive study: some answers, many questions," *Lancet*, vol. 366, no. 9493, pp. 1241–1242, 2005.

[93] J. Lee and M. Reding, "Effects of thiazolidinediones on stroke recovery: a case-matched controlled study," *Neurochemical Research*, vol. 32, no. 4-5, pp. 635–638, 2007.

[94] A. Diab, C. Deng, J. D. Smith, et al., "Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis," *Journal of Immunology*, vol. 168, no. 5, pp. 2508–2515, 2002.

[95] M. Kiaei, K. Kipiani, J. Chen, N. Y. Calingasan, and M. F. Beal, "Peroxisome proliferator-activated receptor-γ agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis," *Experimental Neurology*, vol. 191, no. 2, pp. 331–336, 2005.

[96] R. E. Mrak and G. E. Landreth, "PPARγ, neuroinflammation, and disease," *Journal of Neuroinflammation*, vol. 1, pp. 1–5, 2004.

[97] J. Culman, Y. Zhao, P. Gohlke, and T. Herdegen, "PPAR-γ: therapeutic target for ischemic stroke," *Trends in Pharmacological Sciences*, vol. 28, no. 5, pp. 244–249, 2007.

[98] D. L. Feinstein, "Therapeutic potential of peroxisome proliferator-activated receptor agonists for neurological disease," *Diabetes Technology and Therapeutics*, vol. 5, no. 1, pp. 67–73, 2003.

[99] T. Breidert, J. Callebert, M. T. Heneka, G. Landreth, J. M. Launay, and E. C. Hirsch, "Protective action of the peroxisome proliferator-activated receptor-γ agonist pioglitazone in a mouse model of Parkinson's disease," *Journal of Neurochemistry*, vol. 82, no. 3, pp. 615–624, 2002.

[100] L. Steinman, R. Martin, C. Bernard, P. Conlon, and J. R. Oksenberg, "Multiple sclerosis: deeper understanding of its pathogenesis reveals new targets for therapy," *Annual Review of Neuroscience*, vol. 25, pp. 491–505, 2002.

[101] B. Schütz, J. Reimann, L. Dumitrescu-Ozimek, et al., "The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice," *Journal of Neuroscience*, vol. 25, no. 34, pp. 7805–7812, 2005.

[102] P. N. Pompl, L. Ho, M. Bianchi, T. McManus, W. Qin, and G. M. Pasinetti, "A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis," *The FASEB Journal*, vol. 17, no. 6, pp. 725–727, 2003.

[103] D. M. McTigue, R. Tripathi, P. Wei, and A. T. Lash, "The PPAR γ agonist Pioglitazone improves anatomical and locomotor recovery after rodent spinal cord injury," *Experimental Neurology*, vol. 205, no. 2, pp. 396–406, 2007.

[104] E. J. Park, S. Y. Park, E.-H. Joe, and I. Jou, "15d-PGJ_2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia," *Journal of Biological Chemistry*, vol. 278, no. 17, pp. 14747–14752, 2003.

[105] B. P. Neve, D. Corseaux, G. Chinetti, et al., "PPARα agonists inhibit tissue factor expression in human monocytes and macrophages," *Circulation*, vol. 103, no. 2, pp. 207–212, 2001.

[106] N. F. L. Spencer, M. E. Poynter, S.-Y. Im, and R. A. Daynes, "Constitutive activation of NF-κB in an animal model of aging," *International Immunology*, vol. 9, no. 10, pp. 1581–1588, 1997.

[107] B. Kieć-Wilk, A. Dembińska-Kieć, A. Olszanecka, M. Bodzioch, and K. Kawecka-Jaszcz, "The selected pathophysiological aspects of PPARs activation," *Journal of Physiology and Pharmacology*, vol. 56, no. 2, pp. 149–162, 2005.

[108] B. S. Aspey, S. Ehteshami, C. M. Hurst, S. Pereira, and M. J. G. Harrison, "Effect of bezafibrate on the sequelae of acute experimental focal cerebral ischaemia," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 52, no. 12, pp. 1432–1434, 1989.

[109] F. O'Rourke, N. Dean, N. Akhtar, and A. Shuaib, "Current and future concepts in stroke prevention," *Canadian Medical Association Journal*, vol. 170, no. 7, pp. 1123–1133, 2004.

[110] A. Iwashita, Y. Muramatsu, T. Yamazaki, et al., "Neuroprotective efficacy of the peroxisome proliferator-activated receptor δ-selective agonists in vitro and in vivo," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1087–1096, 2007.

[111] G. Ding, L. Cheng, Q. Qin, S. Frontin, and Q. Yang, "PPARδ modulates lipopolysaccharide-induced TNFα inflammation signaling in cultured cardiomyocytes," *Journal of Molecular and Cellular Cardiology*, vol. 40, no. 6, pp. 821–828, 2006.

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Review Article

Peroxisome Proliferator-Activated Receptors: "Key" Regulators of Neuroinflammation after Traumatic Brain Injury

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Traumatic brain injury is characterized by neuroinflammatory pathological sequelae which contribute to brain edema and delayed neuronal cell death. Until present, no specific pharmacological compound has been found, which attenuates these pathophysiological events and improves the outcome after head injury. Recent experimental studies suggest that targeting peroxisome proliferator-activated receptors (PPARs) may represent a new anti-inflammatory therapeutic concept for traumatic brain injury. PPARs are "key" transcription factors which inhibit NFκB activity and downstream transcription products, such as proinflammatory and proapoptotic cytokines. The present review outlines our current understanding of PPAR-mediated neuroprotective mechanisms in the injured brain and discusses potential future anti-inflammatory strategies for head-injured patients, with an emphasis on the putative beneficial combination therapy of synthetic cannabinoids (e.g., dexanabinol) with PPARα agonists (e.g., fenofibrate).

1. INTRODUCTION

Research efforts in recent years have provided increasing evidence that the intracerebral inflammatory response is in large part responsible for the devastating neuropathological sequelae and poor outcome of traumatic brain injury [1–3]. The extent of brain damage is determined by primary and secondary injury patterns. While the primary injury results from mechanical forces applied to the skull and brain at the time of impact, secondary brain injury occurs as a delayed consequence of trauma [4–7]. Secondary brain injuries are mediated by endogenous pathophysiological processes which lead to an overwhelming neuroinflammation in the injured brain [6, 8–10]. The main risk factors for developing secondary brain injuries are hypoxemia and systemic hypotension which occur frequently in the trauma patient [11, 12]. These conditions contribute to the ischemic brain damage and perpetuate the intracerebral inflammatory reaction through ischemia/reperfusion-mediated mechanisms [13]. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors of the nuclear receptor superfamily which have recently been shown to exert anti-inflammatory properties in acute neurological disorders. These include cerebrovascular stroke, intracerebral hemorrhage, spinal cord injury, and traumatic brain injury [14–21]. The present paper provides an overview on the so far known anti-inflammatory properties of PPARs in brain injury and discusses potential pharmacological properties of PPAR agonists as future neuroprotective agents.

2. BIOLOGICAL FUNCTIONS OF PPARs

PPARs are nuclear membrane-associated transcription factors belonging to the nuclear receptor family [22]. Three isotypes with a differential tissue distribution have been described: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3) [23, 24]. While PPARβ/δ has an ubiquitous expression, PPARα and PPARγ are mainly expressed in tissues

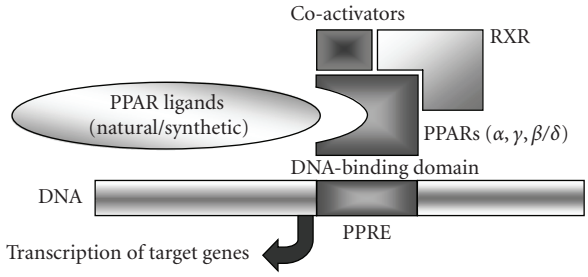

Figure 1: *Mechanism of gene transcription through ligand binding on peroxisome proliferator-activated receptors (PPARs).* In presence of coactivating stimuli, PPARs heterodimerize with retinoid X receptors (RXR) to form active transcription factors. The DNA binding domain on PPAR-RXR heterodimers induces the transcription of target genes by binding to peroxisome proliferator-response elements (PPRE's) which consist of DNA-specific sequences.

with high fatty acid catabolism, such as adipose tissue, liver, kidney, and skeletal muscle [25]. Mechanistically, PPARs are activated by heterodimerization with the retinoid-X receptor (RXR) into biologically active transcription factors. PPAR-RXR heterodimers induce the transcription of candidate genes by binding to so-called peroxisome proliferator-response elements (PPRE's) consisting of DNA-specific sequences (see Figure 1).

PPARs exert a wide variety of physiological functions [24, 26]. They play a central role in the regulation of lipid and lipoprotein metabolism and glucose homeostasis, and have been shown to mediate cellular proliferation and programmed cell death (apoptosis) [27–31]. PPARs have furthermore been involved in bone metabolism and in pathologies of the cardiovascular system and the lung [32–35]. PPARα has been attributed important immunological functions due to its expression on monocytes/macrophages, T cells, and vascular endothelial cells. PPARγ appears to play a crucial role in the regulation of proliferation and differentiation of various cell types. While the biological role of PPARβ/δ has not been defined in detail, recent data imply an antiapoptotic and anti-inflammatory effect after tissue injury, both in vitro and in vivo [29].

From an immunological viewpoint, PPARs have been identified as important regulators of inflammatory gene expression [36–40]. PPARs have also been shown to attenuate adaptive immune responses by inhibiting helper T cell functions and by mediating apoptosis of B cells [41, 42]. PPARs are activated by naturally ocurring fatty acid derivatives, eicosanoides, and by synthetic pharmacological agents, such as fibrates (PPARα) and glitazones (PPARγ) [18, 22, 43]. PPAR ligands have been shown to exert anti-inflammatory activities in various cell types by inhibiting the gene expression for proinflammatory cytokines, metalloproteinases, and hepatic acute-phase proteins.

3. PPARs: "KEY" REGULATORS OF NEUROINFLAMMATION

Mechanistically, the activation of PPARα has been shown to inhibit proinflammatory gene transcription by repressing the central inflammatory transcription factor, nuclear factor-κB (NF-κB) [43–45]. Along with suppression of NF-κB, PPARα acts by inhibition of signal transduction through activator protein-1 (AP-1) signaling [43]. It appears that the inhibitory effect of PPARα on these crucial inflammatory transcription factors creates a negative feedback loop for controlling acute posttraumatic inflammation [44–46]. First in vivo data on the involvement of PPARs in the regulation of inflammation were reported from studies in PPARα knockout mice [47]. Cuzzocrea et al. showed that the targeted deletion of the PPARα gene leads to a significantly increased inflammatory response in different experimental models of acute inflammation outside the central nervous system (CNS) [47]. Within the CNS, the constitutive expression of PPARs has been described for some time [48, 49]. Interestingly, PPAR gene expression was detected not only on vascular endothelial cells in the brain and spinal cord, but also on resident cells in the CNS, such as neurons and glial cells [49].

4. ROLE OF PPARs IN CNS INJURY

In recent years, experimental studies in models of cerebral ischemia/reperfusion injury, ischemic stroke, intracerebral hemorrhage, and spinal cord injury have revealed a crucial role of PPARs in attenuating neuroinflammation and neuronal cell death in the injured CNS (see Table 1) [14–16, 19, 20, 50–52]. PPARα gene-deficient mice (PPAR$\alpha -/-$) were shown to have a significantly worsened neurological outcome, associated with an increased neuroinflammatory response to experimental spinal cord injury, as compared to wild-type littermates [16]. The postulated neuroprotective effects of natural PPARα ligands include the attenuation of polymorphonuclear leukocyte (PMNL) recruitment and associated neurotoxicity, as determined by a significantly reduced expression of myeloperoxidase in the injured spinal cord of PPAR$\alpha -/-$ mice [16]. In addition, tumor necrosis factor (TNF), a "key" mediator of neuroinflammation and neurotoxicity, was shown to be upregulated and associated with neuronal apoptosis in the injured spinal cord of PPAR$\alpha -/-$ mice [16]. In traumatic brain injury, experimental studies in the past decade have shown that TNF is upregulated in the intracranial compartment within a few hours after trauma, and contributes to secondary neuronal injury [53–55]. The deleterious neurotoxic effects were shown to be abrogated by pharmacological inhibition of TNF [56]. Since PPARs inhibit proinflammatory gene transcription by attenuating NF-κB signaling [43–45], the potent PPAR-mediated neuroprotective effects may be dependent on inhibition of NF-κB-dependent proinflammatory cytokines released in the injured brain, such as TNF, interleukin (IL)-1, IL-8, IL-12, and IL-18 [57–61]. The central role of NF-κB signaling in inflammation and oxidative stress explains why PPARs have been considered possible targets for neuroprotection in in-

Table 1: Selected publications on the role of PPARs in CNS injury and inflammation.

Models of CNS injury and neuroinflammation	PPAR isotype	Main findings	Reference no.
Different models of CNS injury	PPARγ	Review on the mechanisms of neuroprotection by PPARγ agonists	Kapadia et al. [20]
Different models of CNS injury	PPARα, PPARγ	Review on pharmacological neuroprotection by PPARs	Bordet et al. [14]
Brain inflammation	PPARγ	Review on regulation of microglial activation by PPARγ agonists	Bernardo and Minghetti [63]
Spinal cord injury	All isotypes	Review on the role of PPAR signal transduction in spinal cord injury	Van Neerven and Mey [15]
Spinal cord injury	PPARα	Experimental model of spinal cord injury in PPARα gene knockout mice. Lack of PPARα leads to worse outcome and increased neuroinflammation.	Genovese et al. [16]
Cerebral ischemia/reperfusion injury	PPARγ	The PPARγ agonists rosiglitazone and pioglitazone exert neuroprotective effects in a rat model of cerebral ischemia/reperfusion injury by reducing neuroinflammation and oxidative stress.	Collino et al. [50]
Intracerebral hemorrhage	PPARγ	PPARγ expressed by microglia and macrophages promotes the resolution of intracerebral hemorrhage and attenuates the neuroinflammatory response.	Zhao et al. [19]
Traumatic brain injury	PPARα	The PPARα agonist fenofibrate reduces brain edema and improves the neurological outcome after experimental fluid percussion brain injury in male Sprague-Dawley rats.	Besson et al. [21]
Traumatic brain injury	PPARα	The PPARα agonist fenofibrate promotes neurological recovery by reducing inflammation and oxidative stress in rat brains after experimental fluid percussion brain injury.	Chen et al. [17]
Neuroinflammation	All isotypes	Review on the interaction between cannabinoids and PPARs as inhibitors of neuroinflammation	Sun and Bennett [83]

flammatory CNS diseases, including traumatic brain injury [14, 20, 62, 63].

5. PHARMACOLOGY OF HEAD INJURY: ARE PPAR-AGONISTS AND CANNABINOIDS THE LONG SOUGHT "GOLDEN BULLET"?

A wide variety of natural and synthetic PPARγ agonists have been described in recent years as regulators of microglial activation and cerebral inflammation [63]. For example, the thiazolinedione pioglitazone has been shown to reduce the extent of neuroinflammation and the severity of disease in experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis (MS) [64, 65]. A recent case report described the impressive clinical improvement of a patient with chronic progressive MS, after a 3-year period of treatment with pioglitazone [66]. This unexpected clinical recovery implies that PPARγ agonists may represent a promising new strategy for attenuating neuroinflammation in patients with CNS autoimmune diseases [62, 63, 67].

In cerebrovascular stroke, the combination therapy of a PPARγ agonist (rosiglitazone) with an antiexcitotoxic glutamate receptor antagonist (MK-801) led to an improved neurological recovery in rats undergoing middle cerebral artery occlusion [18]. A study by another group assessed the therapeutic efficacy of two different PPARγ agonists, rosiglitazone and pioglitazone, in a rat model of cerebral ischemia/reperfusion injury [50]. The authors showed that the pretreatment with either compound led to a significant attenuation of inflammation and oxidative stress in injured rat brains [50].

Pharmacological ligands to PPARα, such as fenofibrate, have also been shown to exert neuroprotective effects in inflammatory CNS conditions. Deplanque et al. demonstrated a significant neuroprotective effect of fenofibrate administration in C57BL/6 mice with cerebrovascular stroke [68]. The authors suggested that PPARα may represent a new pharmacological target to reduce the neuroinflammatory and neuropathological sequelae of cerebrovascular stroke [68].

In traumatic brain injury, the PPARα agonist fenofibrate appears to represent a highly promising new anti-inflammatory compound. Besson et al. assessed the pharmacological role of fenofibrate in a model of experimental fluid-percussion injury in adult male Sprague-Dawley rats [21]. The authors revealed that the administration of fenofibrate during a clinically relevant therapeutic "time window of opportunity" at 1 hour after trauma mediated a significant posttraumatic neuroprotection. This was demonstrated by improved neurological scores in the fenofibrate group at 24 hours and 7 days after trauma, compared to vehicle-treated animals [21]. Morphologically, fenofibrate treatment resulted in significantly decreased extent of brain edema at 24 hours after head injury, compared to the placebo group. The authors furthermore described a marked reduction in intercellular adhesion molecule (ICAM)-1 expression at the protein level by immunohistochemistry in injured rat brain

sections after fenofibrate administration [21]. This finding implies a reduced extent of intracerebral immunoactivation and neuroinflammation in rats treated by the PPARα agonist, compared to vehicle controls.

A more recent follow-up study by the same research group assessed the role of PPARα in modulating the oxidative stress in the injured rat brain [17]. Oxidative stress and ischemia/reperfusion-mediated injuries contribute significantly to the extent of posttraumatic intracerebral inflammation and delayed secondary brain damage after head injury [13, 69, 70]. Pathophysiologically, contused brain areas are surrounded by a penumbra zone which is hypoperfused due to traumatic vascular damage, loss of cerebrovascular autoregulation, and systemic hypotension. After resuscitation, the hypoperfused, ischemic brain areas in the penumbra zone are reperfused, which leads to activation of the complement cascade and of reactive oxygen intermediates by activation of the xanthine oxidase [71, 72]. Oxygen-derived free radicals such as hydroxyl ions, hydrogen peroxide, and superoxide anion induce lipid peroxidation, cell membrane disintegration, and delayed neuronal cell death (see Figure 2). Lipid peroxidation is facilitated in the brain due to its genuine vulnerability to oxidative stress based on specific morphological characteristics, such as a high ratio of "membrane to cytoplasm" and high levels of polyunsaturated fatty acids in the CNS [70]. In addition to reactive oxygen intermediates, the generation of nitric oxide (NO) by inducible NO synthase (iNOS) up-regulation also occurs after head injury and adds to the extent of secondary brain damage [73]. Metabolites emerging from the interaction between superoxide anion and NO, such as the highly reactive oxidant peroxynitrite, have been shown to mediate neurotoxicity and delayed neuronal cell death after traumatic brain injury [74].

The pharmacological administration of the PPARα agonist fenofibrate after experimental fluid-percussion injury resulted in a significant decrease of intracerebral iNOS expression [17]. This was associated with a decreased neuroinflammation in the injured brain and an improved neurological recovery after trauma [17]. These important findings imply that the attenuation of oxidative stress may represent a "key" mechanistic aspect of PPAR-mediated neuroprotection after head injury. The pleiotropic beneficial effects of PPARs in the injured brain, however, are far from being elucidated in detail until present. For example, in contrast to PPARα, no studies have yet been performed to analyze the effect of PPARγ in experimental models of traumatic brain injury (see Table 1).

Despite increasing insights into the pathophysiological mechanisms of posttraumatic neuroinflammation and neurodegeneration, clinical neuroprotection trials have failed to provide a benefit of anti-inflammatory pharmacological strategies with regard to the outcome after head injury [75, 76]. Cannabinoids have recently evolved as a promising new therapeutic avenue for neuroprotection after head injury [77–79]. This group of compounds consists of natural (endocannabinoids) and synthetic ligands, such as dexanabinol (HU-211). The endocannabinoid 2-arachidonoyl glycerol (2-AG) has received increased attention in recent years due to its strong neuroprotective effect after head injury, by inhibition of proinflammatory cytokines, reactive oxygen intermediates, and excitotoxic aminoacids, such as glutamate [80, 81]. The pharmacological agent dexanabinol was shown to mediate neuroprotection by inhibition of TNF production in injured rodent brains [77, 82] and was recently proposed as an effective neuroprotective strategy to reduce the extent of secondary brain injury in humans (see Figure 2) [78, 79]. Dexanabinol (HU-211) is a nonpsychotropic, synthetic cannabinoid which exerts beneficial effects by cytokine inhibition and radical scavenging associated with reduction of brain edema [77–79, 82]. Cannabinoids were attributed a new role as neuroprotective agents by agonistic action to PPARs [83]. The functional interaction between cannabinoids and PPARs was first described based on the finding of oleylethanolamide (OEA), a lipid derivate structurally related to anandamide, as a regulator of feeding behavior in rats through activation of PPARα [68, 84]. Aside from OEA, which is a low-affinity agonist to cannabinoid receptors, other cannabinoids were recently described as PPAR ligands [83]. As such, Δ^9-tetrahydrocannabinol (THC) was found to activate PPARγ in human cell lines [85]. Of particular interest for neuroprotection in traumatic brain injury is the finding that the potent endocannabinoid 2-AG [80, 81] has been found to suppress the proinflammatory cytokine IL-2 through PPARγ signaling, independent of 2-AG binding to cannabinoid re-

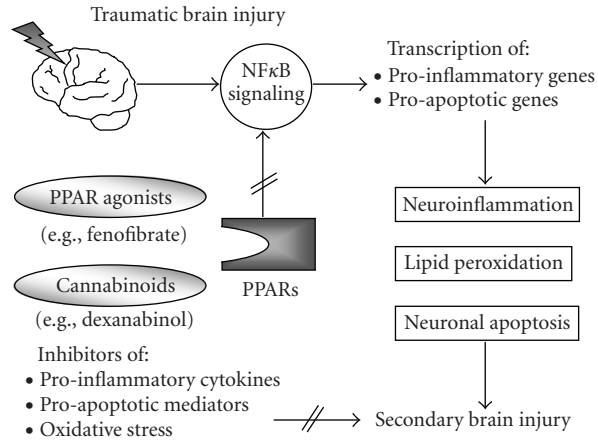

Figure 2: *Working hypothesis of PPAR-mediated mechanisms of neuroprotection after traumatic brain injury*. The neuropathological sequelae of head injury include the posttraumatic activation of NFκB-dependent inflammatory genes. The transcription of neuroinflammatory mediators in the injured brain induces and perpetuates the intracranial inflammatory response and leads to formation of brain edema and adverse outcome. Activation of PPARs by binding of synthetic ligands, such as the PPARα agonist fenofibrate, leads to inhibition of NFκB and of downstream transcribed proinflammatory and proapoptotic mediators. In addition, cannabinoids have a dual neuroprotective function, (1) by acting as ligands to PPARs and (2) by inhibiting "key" mediators of neuroinflammation and apoptosis, such as tumor necrosis factor (TNF). The combination therapy of synthetic PPAR agonists and cannabinoids may represent the long sought pharmacological "golden bullet" for the treatment of traumatic brain injury in the future.

ceptors [86]. Future studies will have to determine whether cannabinoids represent the long sought "golden bullet" for reduction of secondary brain damage after head injury. It seems reasonable to suggest that a combination of neuroprotective cannabinoids, such as dexanabinol, with other potent anti-inflammatory therapeutic agents, such as synthetic PPAR ligands, may represent a promising new therapeutic avenue for improving the outcome of traumatic brain injury.

REFERENCES

[1] T. M. McMillan and G. M. Teasdale, "Death rate is increased for at least 7 years after head injury: a prospective study," *Brain*, vol. 130, no. 10, pp. 2520–2527, 2007.

[2] J. F. Holmes, G. W. Hendey, J. A. Oman, et al., "Epidemiology of blunt head injury victims undergoing ED cranial computed tomographic scanning," *American Journal of Emergency Medicine*, vol. 24, no. 2, pp. 167–173, 2006.

[3] E. M. R. Doppenberg, S. C. Choi, and R. Bullock, "Clinical trials in traumatic brain injury: lessons for the future," *Journal of Neurosurgical Anesthesiology*, vol. 16, no. 1, pp. 87–94, 2004.

[4] R. M. Chesnut, "Care of central nervous system injuries," *Surgical Clinics of North America*, vol. 87, no. 1, pp. 119–156, 2007.

[5] H. Bayir, P. M. Kochanek, and R. S. B. Clark, "Traumatic brain injury in infants and children mechanisms of secondary damage and treatment in the intensive care unit," *Critical Care Clinics*, vol. 19, no. 3, pp. 529–549, 2003.

[6] H. G. Fritz and R. Bauer, "Secondary injuries in brain trauma: effects of hypothermia," *Journal of Neurosurgical Anesthesiology*, vol. 16, no. 1, pp. 43–52, 2004.

[7] P. M. Kochanek, R. S. B. Clark, R. A. Ruppel, et al., "Biochemical, cellular, and molecular mechanisms in the evolution of secondary damage after severe traumatic brain injury in infants and children: lessons learned from the bedside," *Pediatric Critical Care Medicine*, vol. 1, no. 1, pp. 4–19, 2000.

[8] O. I. Schmidt, C. E. Heyde, W. Ertel, and P. F. Stahel, "Closed head injury: an inflammatory disease?" *Brain Research Reviews*, vol. 48, no. 2, pp. 388–399, 2005.

[9] P. F. Stahel, W. R. Smith, and E. E. Moore, "Role of biological modifiers regulating the immune response after trauma," *Injury*, vol. 38, no. 12, pp. 1409–1422, 2007.

[10] B. J. Kelley, J. Lifshitz, and J. T. Povlishock, "Neuroinflammatory responses after experimental diffuse traumatic brain injury," *Journal of Neuropathology and Experimental Neurology*, vol. 66, no. 11, pp. 989–1001, 2007.

[11] P. F. Stahel, W. R. Smith, and E. E. Moore, "Hypoxia and hypotension, the "lethal duo" in traumatic brain injury: implications for prehospital care," *Intensive Care Medicine*, vol. 34, no. 3, pp. 402–404, 2008.

[12] B. J. Eastridge, J. Salinas, J. G. McManus, et al., "Hypotension begins at 110 mm Hg: redefining "hypotension" with data," *Journal of Trauma-Injury Infection & Critical Care*, vol. 63, no. 2, pp. 291–297, 2007, discussion 297–299.

[13] R. R. Leker and E. Shohami, "Cerebral ischemia and trauma—different etiologies yet similar mechanisms: neuroprotective opportunities," *Brain Research Reviews*, vol. 39, no. 1, pp. 55–73, 2002.

[14] R. Bordet, T. Ouk, O. Petrault, et al., "PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases," *Biochemical Society Transactions*, vol. 34, no. 6, pp. 1341–1346, 2006.

[15] S. van Neerven and J. Mey, "RAR/RXR and PPAR/RXR signaling in spinal cord injury," *PPAR Research*, vol. 2007, Article ID 29275, 14 pages, 2007.

[16] T. Genovese, E. Mazzon, R. Di Paola, et al., "Role of endogenous ligands for the peroxisome proliferators activated receptors α in the secondary damage in experimental spinal cord trauma," *Experimental Neurology*, vol. 194, no. 1, pp. 267–278, 2005.

[17] X. R. Chen, V. C. Besson, B. Palmier, Y. Garcia, M. Plotkine, and C. Marchand-Leroux, "Neurological recovery-promoting, anti-inflammatory, and anti-oxidative effects afforded by fenofibrate, a PPAR α agonist, in traumatic brain injury," *Journal of Neurotrauma*, vol. 24, no. 7, pp. 1119–1131, 2007.

[18] M. Allahtavakoli, A. Shabanzadeh, A. Roohbakhsh, and A. Pourshanazari, "Combination therapy of rosiglitazone, a peroxisome proliferator-activated receptor-γ ligand, and NMDA receptor antagonist (MK-801) on experimental embolic stroke in rats," *Basic & Clinical Pharmacology & Toxicology*, vol. 101, no. 5, pp. 309–314, 2007.

[19] X. Zhao, G. Sun, J. Zhang, et al., "Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor γ in microglia/macrophages," *Annals of Neurology*, vol. 61, no. 4, pp. 352–362, 2007.

[20] R. Kapadia, J.-H. Yi, and R. Vemuganti, "Mechanisms of anti-inflammatory and neuroprotective actions of PPAR-γ agonists," *Frontiers in Bioscience*, vol. 13, pp. 1813–1826, 2008.

[21] V. C. Besson, X. R. Chen, M. Plotkine, and C. Marchand-Verrecchia, "Fenofibrate, a peroxisome proliferator-activated receptor α agonist, exerts neuroprotective effects in traumatic brain injury," *Neuroscience Letters*, vol. 388, no. 1, pp. 7–12, 2005.

[22] T. M. Willson, P. J. Brown, D. D. Sternbach, and B. R. Henke, "The PPARs: from orphan receptors to drug discovery," *Journal of Medicinal Chemistry*, vol. 43, no. 4, pp. 527–550, 2000.

[23] L. Michalik and W. Wahli, "Peroxisome proliferator-activated receptors: three isotypes for a multitude of functions," *Current Opinion in Biotechnology*, vol. 10, no. 6, pp. 564–570, 1999.

[24] S. A. Kliewer, H. E. Xu, M. H. Lambert, and T. M. Willson, "Peroxisome proliferator-activated receptors: from genes to physiology," *Recent Progress in Hormone Research*, vol. 56, pp. 239–263, 2001.

[25] K. Schoonjans, J. Peinado-Onsurbe, A. M. Lefebvre, et al., "PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene," *EMBO Journal*, vol. 15, no. 19, pp. 5336–5348, 1996.

[26] B. P. Kota, T. H.-W. Huang, and B. D. Roufogalis, "An overview on biological mechanisms of PPARs," *Pharmacological Research*, vol. 51, no. 2, pp. 85–94, 2005.

[27] S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann, "A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation," *Cell*, vol. 83, no. 5, pp. 813–819, 1995.

[28] R. P. Brun, P. Tontonoz, B. M. Forman, et al., "Differential activation of adipogenesis by multiple PPAR isoforms," *Genes and Development*, vol. 10, no. 8, pp. 974–984, 1996.

[29] N. S. Tan, L. Michalik, N. Noy, et al., "Critical roles of PPAR β/δ in keratinocyte response to inflammation," *Genes & Development*, vol. 15, no. 24, pp. 3263–3277, 2001.

[30] S. Kersten, "Peroxisome proliferator activated receptors and

lipoprotein metabolism," *PPAR Research*, vol. 2008, Article ID 132960, 11 pages, 2008.

[31] G. Martinasso, M. Oraldi, A. Trombetta, et al., "Involvement of PPARs in cell proliferation and apoptosis in human colon cancer specimens and in normal and cancer cell lines," *PPAR Research*, vol. 2007, Article ID 93416, 9 pages, 2007.

[32] B. Lecka-Czernik, "PPARs and bone metabolism," *PPAR Research*, vol. 2006, Article ID 18089, 1 page, 2006.

[33] S. Z. Duan, C. Y. Ivashchenko, M. G. Usher, and R. M. Mortensen, "PPAR-γ in the cardiovascular system," *PPAR Research*, vol. 2008, Article ID 745804, 10 pages, 2008.

[34] R. Di Paola and S. Cuzzocrea, "Peroxisome proliferator-activated receptors and acute lung injury," *PPAR Research*, vol. 2007, Article ID 63745, 8 pages, 2007.

[35] T. H.-W. Huang, V. Razmovski-Naumovski, B. P. Kota, D. S.-H. Lin, and B. D. Roufogalis, "The pathophysiological function of peroxisome proliferator-activated receptor-γ in lung-related diseases," *Respiratory Research*, vol. 6, Article ID 102, 9 pages, 2005.

[36] P. Delerive, J. C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors in inflammation control," *Journal of Endocrinology*, vol. 169, no. 3, pp. 453–459, 2001.

[37] A. Cabrero, J. C. Laguna, and M. Vazquez, "Peroxisome proliferator-activated receptors and the control of inflammation," *Current Drug Targets—Inflammation & Allergy*, vol. 1, no. 3, pp. 243–248, 2002.

[38] G. Chinetti, J. C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation," *Inflammation Research*, vol. 49, no. 10, pp. 497–505, 2000.

[39] R. S. B. Clark, "The role of PPARs in inflammation and immunity," *Journal of Leukocyte Biology*, vol. 71, no. 3, pp. 388–400, 2002.

[40] R. A. Daynes and D. C. Jones, "Emerging roles of PPARs in inflammation and immunity," *Nature Reviews Immunology*, vol. 2, no. 10, pp. 748–759, 2002.

[41] R. S. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPAR γ and immunoregulation: PPAR γ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[42] D. M. Ray, F. Akbiyik, S. H. Bernstein, and R. P. Phipps, "CD40 engagement prevents peroxisome proliferator-activated receptor γ agonist-induced apoptosis of B lymphocytes and B lymphoma cells by an NF-κB-dependent mechanism," *Journal of Immunology*, vol. 174, pp. 4060–4069, 2005.

[43] B. Staels and J.-C. Fruchart, "Therapeutic roles of peroxisome proliferator-activated receptor agonists," *Diabetes*, vol. 54, no. 8, pp. 2460–2470, 2005.

[44] M. E. Poynter and R. A. Daynes, "Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor-κB signaling, and reduces inflammatory cytokine production in aging," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32833–32841, 1998.

[45] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[46] L. Michalik and W. Wahli, "Involvement of PPAR nuclear receptors in tissue injury and wound repair," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 598–606, 2006.

[47] S. Cuzzocrea, E. Mazzon, R. Di Paola, et al., "The role of the peroxisome proliferator-activated receptor-α (PPAR-α) in the regulation of acute inflammation," *Journal of Leukocyte Biology*, vol. 79, no. 5, pp. 999–1010, 2006.

[48] T. E. Cullingford, K. Bhakoo, S. Peuchen, C. T. Dolphin, R. Patel, and J. B. Clark, "Distribution of mRNAs encoding the peroxisome proliferator-activated receptor α, β, and γ and the retinoid X receptor α, β, and γ in rat central nervous system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1366–1375, 1998.

[49] S. Moreno, S. Farioli-Vecchioli, and M. P. Cerù, "Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS," *Neuroscience*, vol. 123, no. 1, pp. 131–145, 2004.

[50] M. Collino, M. Aragno, R. Mastrocola, et al., "Modulation of the oxidative stress and inflammatory response by PPAR-γ agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion," *European Journal of Pharmacology*, vol. 530, no. 1-2, pp. 70–80, 2006.

[51] S.-D. Chen, H.-Y. Wu, D.-I. Yang, et al., "Effects of rosiglitazone on global ischemia-induced hippocampal injury and expression of mitochondrial uncoupling protein 2," *Biochemical and Biophysical Research Communications*, vol. 351, no. 1, pp. 198–203, 2006.

[52] Y. Luo, W. Yin, A. P. Signore, et al., "Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-γ agonist rosiglitazone," *Journal of Neurochemistry*, vol. 97, no. 2, pp. 435–448, 2006.

[53] E. Shohami, M. Novikov, R. Bass, A. Yamin, and R. Gallily, "Closed head injury triggers early production of TNF-α and IL-6 by brain tissue," *Journal of Cerebral Blood Flow & Metabolism*, vol. 14, pp. 615–619, 1994.

[54] L. Fan, P. R. Young, F. C. Barone, G. Z. Feuerstein, D. H. Smith, and T. K. McIntosh, "Experimental brain injury induces differential expression of tumor necrosis factor-α mRNA in the CNS," *Molecular Brain Research*, vol. 36, no. 2, pp. 287–291, 1996.

[55] S. M. Knoblach, L. Fan, and A. I. Faden, "Early neuronal expression of tumor necrosis factor-α after experimental brain injury contributes to neurological impairment," *Journal of Neuroimmunology*, vol. 95, no. 1-2, pp. 115–125, 1999.

[56] E. Shohami, R. Bass, D. Wallach, A. Yamin, and R. Gallily, "Inhibition of tumor necrosis factor α (TNFα) activity in rat brain is associated with cerebroprotection after closed head injury," *Journal of Cerebral Blood Flow & Metabolism*, vol. 16, pp. 378–384, 1996.

[57] E. Shohami, I. Ginis, and J. M. Hallenbeck, "Dual role of tumor necrosis factor α in brain injury," *Cytokine and Growth Factor Reviews*, vol. 10, no. 2, pp. 119–130, 1999.

[58] A. Gopcevic, B. Mazul-Sunko, J. Marout, et al., "Plasma interleukin-8 as a potential predictor of mortality in adult patients with severe traumatic brain injury," *Tohoku Journal of Experimental Medicine*, vol. 211, no. 4, pp. 387–393, 2007.

[59] P. F. Stahel, T. Kossmann, H. Joller, O. Trentz, and M. C. Morganti-Kossmann, "Increased interleukin-12 levels in human cerebrospinal fluid following severe head trauma," *Neuroscience Letters*, vol. 249, no. 2-3, pp. 123–126, 1998.

[60] U. Felderhoff-Mueser, O. I. Schmidt, A. Oberholzer, C. Buhrer, and P. F. Stahel, "IL-18: a key player in neuroinflammation and neurodegeneration?" *Trends in Neurosciences*, vol. 28, no. 9, pp. 487–493, 2005.

[61] S.-M. Lucas, N. J. Rothwell, and R. M. Gibson, "The role of inflammation in CNS injury and disease," *British Journal of Pharmacology*, vol. 147, supplement 1, pp. S232–S240, 2006.

[62] R. Bordet, P. Gele, P. Duriez, and J.-C. Fruchart, "PPARs: a new target for neuroprotection," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 77, no. 3, pp. 285–286, 2006.

[63] A. Bernando and L. Minghetti, "PPAR-γ agonists as regulators of microglial activation and brain inflammation," *Current Pharmaceutical Design*, vol. 12, no. 1, pp. 93–109, 2006.

[64] M. Niino, K. Iwabuchi, S. Kikuchi, et al., "Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-γ," *Journal of Neuroimmunology*, vol. 116, no. 1, pp. 40–48, 2001.

[65] C. Natarajan and J. J. Bright, "Peroxisome proliferator-activated receptor-γ agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation," *Genes & Immunity*, vol. 3, no. 2, pp. 59–70, 2002.

[66] H. A. Pershadsingh, M. T. Heneka, R. Saini, N. M. Amin, D. J. Broeske, and D. L. Feinstein, "Effect of pioglitazone treatment in a patient with secondary multiple sclerosis," *Journal of Neuroinflammation*, vol. 1, article 3, pp. 1–4, 2004.

[67] R. E. Mrak and G. E. Landreth, "PPARγ, neuroinflammation, and disease," *Journal of Neuroinflammation*, vol. 1, article 5, pp. 1–3, 2004.

[68] D. Deplanque, P. Gelé, O. Pétrault, et al., "Peroxisome proliferator-activated receptor-α activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment," *Journal of Neuroscience*, vol. 23, no. 15, pp. 6264–6271, 2003.

[69] O. I. Schmidt, M. Infanger, C. E. Heyde, W. Ertel, and P. F. Stahel, "The role of neuroinflammation in traumatic brain injury," *European Journal of Trauma*, vol. 30, no. 3, pp. 135–149, 2004.

[70] P. H. Evans, "Free radicals in brain metabolism and pathology," *British Medical Bulletin*, vol. 49, no. 3, pp. 577–587, 1993.

[71] P. F. Stahel, M. C. Morganti-Kossmann, and T. Kossmann, "The role of the complement system in traumatic brain injury," *Brain Research Reviews*, vol. 27, no. 3, pp. 243–256, 1998.

[72] A. L. D'Ambrosio, D. J. Pinsky, and E. S. Connolly, "The role of the complement cascade in ischemia/reperfusion injury: implications for neuroprotection," *Molecular Medicine*, vol. 7, no. 6, pp. 367–382, 2001.

[73] L. Cherian, J. C. Goodman, and C. S. Robertson, "Brain nitric oxide changes after controlled cortical impact injury in rats," *Journal of Neurophysiology*, vol. 83, no. 4, pp. 2171–2178, 2000.

[74] E. D. Hall, N. C. Kupina, and J. S. Althaus, "Peroxynitrite scavengers for the acute treatment of traumatic brain injury," *Annals of the New York Academy of Sciences*, vol. 890, no. 1, pp. 462–468, 1999.

[75] R. K. Narayan, M. E. Michel, B. Ansell, et al., "Clinical trials in head injury," *Journal of Neurotrauma*, vol. 19, no. 5, pp. 503–557, 2002.

[76] T. J. DeGraba and L. C. Pettigrew, "Why do neuroprotective drugs work in animals but not humans?" *Neurologic Clinics*, vol. 18, no. 2, pp. 475–493, 2000.

[77] E. Shohami, R. Gallily, R. Mechoulam, R. Bass, and T. Ben-Hur, "Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-α inhibitor and an effective neuroprotectant," *Journal of Neuroimmunology*, vol. 72, no. 2, pp. 169–177, 1997.

[78] R. Mechoulam, D. Panikashvili, and E. Shohami, "Cannabinoids and brain injury: therapeutic implications," *Trends in Molecular Medicine*, vol. 8, no. 2, pp. 58–61, 2002.

[79] H. Bayir, R. S. B. Clark, and P. M. Kochanek, "Promising strategies to minimize secondary brain injury after head trauma," *Critical Care Medicine*, vol. 31, supplement 1, pp. S112–S117, 2003.

[80] D. Panikashvili, C. Simeonidou, S. Ben-Shabat, L. Hanus, A. Breuer, R. Mechoulam, and E. Shohami, "An endogenous cannabinoid (2-AG) is neuroprotective after brain injury," *Nature*, vol. 413, pp. 527–531, 2001.

[81] R. Mechoulam and E. Shohami, "Endocannabinoids and traumatic brain injury," *Molecular Neurobiology*, vol. 36, no. 1, pp. 68–74, 2007.

[82] E. Shohami, M. Novikov, and R. Mechoulam, "A nonpsychotropic cannabinoid, HU-211, has cerebroprotective effects after closed head injury in the rat," *Journal of Neurotrauma*, vol. 10, no. 2, pp. 109–119, 1993.

[83] Y. Sun and A. Bennett, "Cannabinoids: a new group of agonists of PPARs," *PPAR Research*, vol. 2007, Article ID 23513, 7 pages, 2007.

[84] J. Fu, F. Oveisi, S. Gaetani, E. Lin, and D. Piomelli, "Oleoylethanolamide, an endogenous PPAR-α agonist, lowers body weight and hyperlipidemia in obese rats," *Neuropharmacology*, vol. 48, no. 8, pp. 1147–1153, 2005.

[85] S. E. O'Sullivan, E. J. Tarling, A. J. Bennett, D. A. Kendall, and M. D. Randall, "Novel time-dependent vascular actions of Δ^9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor γ," *Biochemical and Biophysical Research Communications*, vol. 337, no. 3, pp. 824–831, 2005.

[86] C. E. Rockwell, N. T. Snider, J. T. Thompson, J. P. Vanden Heuvel, and N. E. Kaminski, "Interleukin-2 suppression by 2-arachidonyl glycerol is mediated through peroxisome proliferator-activated receptor γ independently of cannabinoid receptors 1 and 2," *Molecular Pharmacology*, vol. 70, no. 1, pp. 101–111, 2006.

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Review Article

Peroxisome Proliferator-Activated Receptor-γ in Amyotrophic Lateral Sclerosis and Huntington's Disease

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Amyotrophic lateral sclerosis (ALS) is a debilitating and one of the most common adult-onset neurodegenerative diseases with the prevalence of about 5 per 100 000 individuals. It results in the progressive loss of upper and lower motor neurons and leads to gradual muscle weakening ultimately causing paralysis and death. ALS has an obscure cause and currently no effective treatment exists. In this review, a potentially important pathway is described that can be activated by peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists and has the ability to block the neuropathological damage caused by inflammation in ALS and possibly in other neudegenerative diseases like Huntington's disease (HD). Neuroinflammation is a common pathological feature in neurodegenerative diseases. Therefore, PPAR-γ agonists are thought to be neuroprotective in ALS and HD. We and others have tested the neuroprotective effect of pioglitazone (Actos), a PPAR-γ agonist, in G93A SOD1 transgenic mouse model of ALS and found significant increase in survival of G93A SOD1 mice. These findings suggest that PPAR-γ may be an important regulator of neuroinflammation and possibly a new target for the development of therapeutic strategies for ALS. The involvement of PPAR-γ in HD is currently under investigation, one study finds that the treatment with rosiglitazone had no protection in R6/2 transgenic mouse model of HD. PPAR-γ coactivator-1α (PGC-1α) is a transcriptional coactivator that works together with combination of other transcription factors like PPAR-γ in the regulation of mitochondrial biogenesis. Therefore, PPAR-γ is a possible target for ALS and HD as it functions as transcription factor that interacts with PGC-1α. In this review, the role of PPAR-γ in ALS and HD is discussed based on the current literature and hypotheses.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily which includes PPAR-γ, PPAR-α, and PPAR-β/δ. PPAR-γ is the most studied receptor and has two isoforms produced due to alternative splicing and alternate translation initiation: PPAR-γ_1 and PPAR-γ_2 [1, 2]. Another ligand-activated transcription factor is retinoid-X receptor from the same superfamily that forms heterodimeric complexes with PPARs in response to ligand binding. These heterodimeric complexes bind to the cis-acting sequences that are also called peroxisome proliferators response element (PPRE) on DNA to activate or inactivate the transcription of target genes (for further details see [3–5]).

PPARs are ligand-dependent transcription factors that bind to specific PPREs and enhance the expression of regulated genes [6]. PPARs regulate the expression of target genes, in particular those associated with lipid metabolism [7–9]. PPAR isotypes appear to exhibit distinct patterns of tissue distribution and differ considerably in their ligand binding domains, implying that they possibly perform different functions in different cell types [2, 10–13]. PPAR-α is expressed in high levels in hepatocytes, entrocytes, and kidney [12]. PPAR-α is implicated to be responsible for the peroxisome proliferator-induced pleiotropic responses [14]. PPAR-α and δ appear primarily to stimulate oxidative lipid metabolism, while PPAR-γ is principally involved in the cellular assimilation of lipids via anabolic pathways. Recently, other functions for PPAR-γ are described, such as neuroprotection in ischemia [15] and its

effect on spinal cord injury (SCI) [16] (also for review see [17]).

PPAR-γ has been demonstrated to be involved in adipogenesis and differentiation, and its involvement in other tissues specifically in central nervous system is rapidly emerging [16, 18–20]. PPAR-γ is shown to have a vital role in adipocyte differentiation both in vivo and in vitro [1, 21, 22]. Recent studies demonstrate PPAR-γ agonists to prevent inflammation and neuronal death after focal cerebral ischemia in rodents [15, 23–25]. Thiazolidinediones (TZDs) are potent synthetic agonists of PPAR-γ shown to induce neuroprotection after cerebral ischemia by blocking inflammation. In a recent study, pioglitazone and rosiglitazone (Figure 1(a)) treatment in SCI in adult rat significantly decreased the lesion size, motor neuron loss, myelin loss, as well as astrogliosis and microglial activation due to SCI [16]. These TZDs significantly enhanced the motor function recovery after SCI. The beneficial and protective lipid-independent effects of TZDs are the anti-inflammatory capacities of PPAR-γ [4]. TZDs inhibit the expression of various inflammatory proteins like inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), and matrix metalloproteinase-9 (MMP-9) in macrophages [26] and are beneficial in disorders such as inflammatory bowel disease [27]. These inflammatory molecules are shown to be neurotoxic in models of neurodegenerative diseases, for example, in ALS [28–30]. Several anti-inflammatory mechanisms have been suggested, including inhibition of nuclear factor kappa B (NF-κB), activator protein-1 (AP-1), in addition to signal transducers and activators of transcription (STAT) transcription factors by PPAR-γ [31]. Although nuclear receptors repress target genes in the absence of ligand by recruiting corepressors, the molecular mechanism for transcriptional repression by nuclear receptors in response to the binding of ligands await further research. It is possible that PPAR-γ is involved in the reciprocal inhibition of differential transcription systems through limited availability of shared cofactors. Recently, an alternative mechanism suggested that a functionally distinct pool of PPAR-γ is susceptible to ligand-dependent sumoylation (covalent attachment of small ubiquitin-like modifier) at lysine 365, leading to recruitment and stabilization of nuclear corepressor (N-CoR) complexes at the promoters of proinflammatory genes thereby repressing them [32].

2. PPAR-γ AND AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a devastating fatal neurodegenerative disorder characterized by a loss of upper and lower motor neurons. Oxidative stress, mitochondrial dysfunction, and neuroinflammation have been implicated in ALS pathogenesis (Figure 1(b)). PPARs, in particular PPAR-γ, may be a major signaling pathway involved in neuroinflammation in ALS. The activation or inactivation of PPAR-γ could provide a viable and promising approach to understand the mechanism of neuroinflammation in ALS (Figure 1(b)). Since neuroinflammatory pathway has become one of the hallmarks of ALS [29, 33, 34], therefore, blockage of neuroinflammation is of great interest because of the potential efficacy in ALS patients. PPAR-γ has been identified as a key regulatory factor in the modulation of target genes with PPAR response element (PPRE) in their promoters, including those encoding for inflammation (iNOS, NF-κB, COX-2), oxidative stress, and apoptosis. Synthetic PPAR-γ agonists developed in the past 25 years that are used primarily as antidiabetic drugs are suitable candidates and are indispensable to study the role of PPAR-γ in ALS which may potentially lead to beneficial effects in ALS patients.

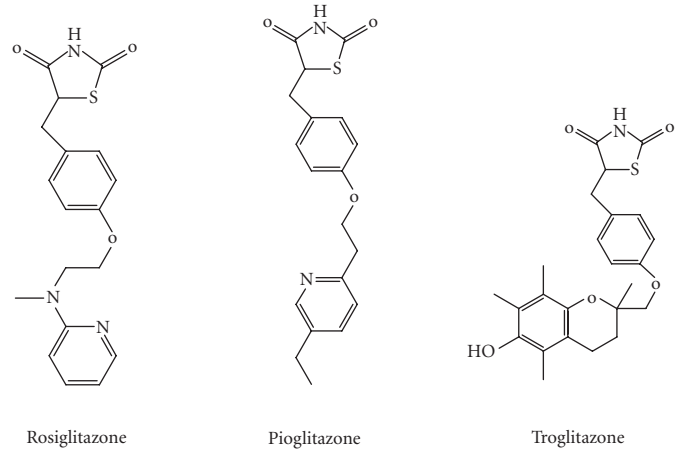
Previous studies have shown the protective effect of PPAR-γ agonists in many experimental models such as in experimental autoimmune encephalomyelitis (EAE) [35], cytokine-induced apoptotic cell death of cerebellar granule cells in vitro and in vivo, and against glutamate-induced cell death in mixed cortical neurons and glia cocultures [36, 37]. Additionally, PPAR-γ agonists are reported to be neuroprotective in tyrosine hydroxylase positive neurons in substantia nigra when exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [18, 19].

3. PIOGLITAZONE IS NEUROPROTECTIVE IN ALS

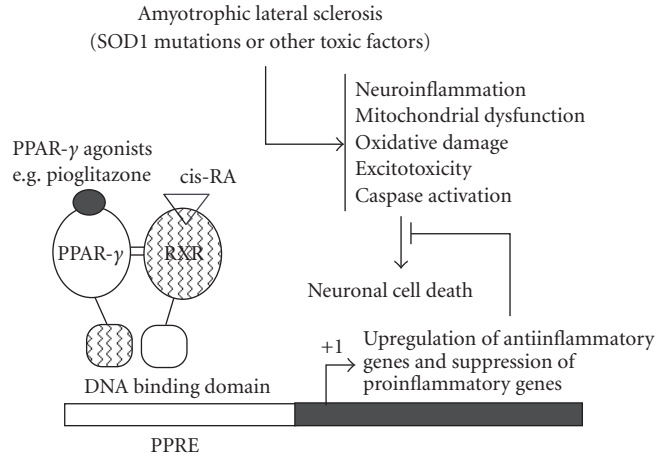
We have tested the neuroprotective effect of pioglitazone in transgenic G93A SOD1 mouse model of ALS and showed that pioglitazone treatment improved motor performance, delayed weight loss, attenuated motor neuron loss, and significantly increased survival by delaying the onset of ALS [38]. Our results also show that pioglitazone treatment reduced microglial activation and gliosis in the spinal cord as assessed by immunohistochemical staining for CD40 (microglia marker) and GFAP (astrocyte marker), respectively. Furthermore, we showed that pioglitazone treatment reduced iNOS, NF-κB, and 3-nitotyrosine immunoreactivity in the spinal cord of G93A transgenic mice.

Our findings were also confirmed by another study on the effect of pioglitazone treatment in G93A SOD1 transgenic mouse model of ALS [39]. In this study, PPAR-γ agonist treatment improved survival, muscle strength, and weight loss in ALS mice. Quantification of motor neuron loss was performed at 90 days of age where approximately 30% of motor neurons were lost in G93A mice spinal cord. Pioglitazone treatment completely prevented this motor neuron loss in the spinal cord of G93A mice. They also showed significant reduction in microglial activation as well as reduction in the expression of COX-2 and iNOS [39].

Further evidence in the modulation of proinflammatory markers by pioglitazone were reported by Schütz et al. which suggests that mRNA levels of two cytokine suppressor genes, suppressor of cytokine signaling 1 and 3 (SOCS-1 and -3), were increased as assessed by semiquantitative RT-PCR [39]. Others have reported similar increase in SOCS-1 and -3 in response to TZDs in microglia and astrocytes in vitro [40]. The increase in SOCS-1 and -3 is implicated with the inhibition of Janus kinase-signal transducer and activator of transcription (JAK-STAT) in inflammatory signal transduction. Other studies using PPAR-γ agonists suggest that the mechanism of actions are also by induction of neuroprotective genes such as heat shock proteins [16]. Recently, Xu and Drew demonstrated that PPAR-γ agonists suppress cytokines like IL-12 family in EAE, an experimental model of multiple sclerosis,



(a)



(b)

Figure 1: (a) Structure of PPAR agonists, (b) schematic diagrams linking mechanisms of neuronal cell death in ALS and a representation of PPAR-γ activation. The mechanisms and pathways implicated in the pathogenesis of ALS that lead to the demise of motor neurons are multiple. Activation of PPAR-γ by pioglitazone has the potential to block inflammatory pathway via the upregulation of anti-inflammatory genes and downregulation of proinflammatory genes. The transcription of PPAR-γ target gene regulation occurs when ligand binds to PPAR-γ and PPAR-γ-RXR heterodimers formed, then it binds to PPRE of DNA of target gene.

when treated with 15-d-PGJ_2 and rosiglitazone [35]. These studies provide evidence that PPAR-γ agonist responses are originating from activated glial cells in central nervous system. PPAR agonists are shown to modulate microglia and astrocytes in central nervous system diseases as these cells are chronically activated and thought to contribute to neuroinflammation with pathological abnormalities in degenerative diseases [41]. Pioglitazone treatment in G93A mice showed reduction in gliosis which is another experimental evidence that PPAR-γ acts on glial cells in CNS [38]. The action of PPAR-γ in neuronal cells needs to be studied.

The preliminary reports on the neuroprotective role of PPAR-γ agonist in transgenic mouse model of ALS and other experimental animal models could potentially be a foundation for new series of studies to understand the mechanism and molecular details of PPARs and their role in protecting motor neurons from inflammatory damages in ALS (Figure 1(b)). The mechanisms of how PPAR-γ agonists induce neuroprotection by blocking neuroinflammation is not fully understood and further information on the molecular details of PPAR-γ in neuroinflammatory pathways will provide crucial insights on the role of PPAR-γ in ALS and other neurodegenerative diseases.

4. MITOCHONDRIAL DYSFUNCTION IN ALS

Mitochondrial compromise in ALS is substantiated by reports of changes in their structure, number, and localization in motor neurons and skeletal muscle, in familial and sporadic ALS patients [42]. Other studies reported the potential involvement of mitochondria in the pathogenesis of ALS as mitochondrial abnormalities were found in proximal axons, anterior horn of ALS spinal cords [43]. Additionally, defects in respiratory chain complexes have been detected in postmortem muscle and spinal cord of ALS patients. Based on the evidence of mitochondrial dysfunction in FALS-SOD1, it is

hypothesized that mutant SOD1 may directly damage mitochondrial function and integrity. Several studies have shown that transgenic mice overexpressing human G93A SOD1 that display most of the ALS symptoms and pathologies have mitochondrial dysfunction. More importantly, several studies suggests that mitochondrial abnormalities occur long before disease onset [44]. Kong and Xu found massive mitochondrial degeneration in motor neurons that are on the brink of dying and even vacuolar and swollen mitochondria are found near motor neuron cell debris in G93A mice [45]. These observations suggest that mitochondrial abnormalities may trigger the onset of ALS. Recently, we and others have shown that wild type and mutant SOD1 are found within mitochondrion which was known to be a cytosolic enzyme [46, 47]. How SOD1 is interacting with mitochondria is unclear and it is being actively investigated. The toxic action of mutant SOD1 in and out of mitochondria could be partly explained as follows. (i) Mutant but not wild type SOD1 binds to heat shock proteins causing an inhibition of chaperon activities. Both mutant and wild type SOD1 bind to antiapoptotic protein Bcl-2, on the outer mitochondrial membrane, blocking its antiapoptotic activity [42]. (ii) The presence of mutant SOD1 in the mitochondria leads to formation of SOD1 aggregates, entrapping Bcl-2, blocking protein importation to mitochondria which may trigger neuronal cell death due to mitochondrial dysfunction [42].

Since PGC-1α is known to coordinate mitochondrial biogenesis and regulates mitochondrial function, it is possible to predict that PGC-1α could play an important role in ALS. Impairment of PGC-1α could contribute to mitochondrial dysfunction in ALS. To date, there is no published data on the role of PGC-1α or its expression in the transgenic mouse model of ALS or human ALS postmortem tissues. However, there are reports on the altered or impaired expression of genes in ALS that some of them fit in the PGC-1α target genes category [29, 48], suggesting that there may be a prominent role for PGC-1α translational machinery in ALS.

Since PGC-1α is a PPAR-γ coactivator, it is possible that PPAR-γ agonists may be able to activate PGC-1α and also the PGC-1α target genes. Like in HD, a reduction of PGC-1α and its target genes expression is attributed to mutant huntingtin, similarly mutant SOD1 could impair PGC-1α and expression of its target genes in ALS. Whether mutant SOD1 can impair PPAR-γ is yet to be determined. Future studies on PGC-1α and PPAR-γ in ALS patients and transgenic mice will shed some lights on these pathways in disease development.

5. PPAR-γ AND HUNTINGTON'S DISEASE

Huntington's disease is an autosomal dominant, fatal neurogenetic disease that affects approximately 1 in 10,000 people [49]. The etiology of HD is shown to be the unstable CAG repeat expansion in the huntingtin gene on chromosome 4 resulting in polyglutamine expansion in huntingtin protein. The polyglutamine expansion causes the aggregation of huntingtin protein and formation of neuronal inclusion bodies as reviewed by Ortega et al. [50]. Mitochondrial dysfunction has been implicated in HD since defects in electron transport chain complexes are evident in several tissues from HD patients and transgenic mouse models of HD [51–55]. The mechanisms for this mitochondrial dysfunction are actively been studied and in spite of some new and novel discoveries and hypotheses, it is not fully understood how mitochondrial dysfunction and oxidative stress and expansion of unstable CAG repeats in huntingtin gene cause HD. Recent reports show that mutant huntingtin interferes with transcriptional PPAR-γ coactivator-1α (PGC-1α) causing impairment on its function in HD, suggesting that mutant huntingtin plays a role in the dysregulation of PGC-1α-mediated transcription and activity, impairing mitochondrial function, and leading to HD pathogenesis [56–58]. Weydt et al. found that PGC-1α target genes (NDUFS3, CYCS, COX6A1, NDUFB5, ACADM, TFAM, and LDHB) had reduced expression in HD patient and mouse striatum [27]. They also found that HD mice brain mitochondria show reduced oxygen consumption rates. An interesting finding was that the PGC-1α and uncoupling protein 1 (UCP-1) circuit was found to be disrupted in the brown adipose tissue (BAT) of HD transgenic mice. This was discovered when HD mice challenged with cold. As in mammals, after cold is sensed in the hypothalamus, an increase in sympathetic tone in the periphery ensues. In rodents, BAT is the tissue that responds to cold. In HD and wild type mice challenged with cold, PGC-1α expression increased, but in HD mice UCP-1, expression was not upregulated. However, they showed that PGC-1α expression is decreased in the striatum of human HD. They also examined the expression of unclear hormone receptors (PPAR-α, RXR-α) and transcription factors (NRF-1) that known to rely upon PGC-1α for target gene activation, these genes were upregulated, suggesting possible compensatory upregulation of PGC-1α-dependent transcription factors in human HD caudate. Weydt et al. study proposes that based on the evidence for impaired energy production and/or impaired responses to oxidative stress, evaluation of metabolic processes occurring in nonneuronal tissues in the periphery may yield factors and pathways that contribute to neurodegenerative diseases. Weydt et al. and Cui et al. studies provide further support that the reduction of PGC-1α and its target genes in HD striatum are caused by mutant huntingtin. Weydt et al. stated from personal communication with J. Boats and R.E. Hughes that their yeast two-hybrid screen identified that PPAR-γ is a huntingtin interactor, and the interaction was validated for its biological significance by demonstrating an effect of PPAR-γ dosage upon HD neurodegeneration in the fly eye [27].

Increased levels of iNOS in HD [59], elevated oxidative damage products such as malondialdehyde, 8-hydroxydeoxyguanosine, 3-nitrotyrosine, and hemeoxygenase in areas of degeneration in HD brain, and increased free radical production in animal models, indicate the involvement of oxidative stress in HD [60]. This important pathway has great promise and must be explored in order to understand the role of PPAR-γ and to identify new therapeutic targets for HD.

Rosiglitazone (a PPAR-γ agonist) that induces sensitization to insulin was tested in R6/2 transgenic mouse model of HD for the treatment of atypical diabetes in these mice

[61]. The effect of glibenclamide (a sulfonylurea) that depolarizes pancreatic beta cells by blocking ATP-sensitive potassium channels to induce exocytosis of insulin leading to increase in insulin levels was also tested in R6/2 mice. Chronic treatment with these two drugs, singly or in combination, did not change the course of diabetes or survival, weight loss of R6/2 mice. In their paper, general characteristics of diabetes in R6/2 mouse model of HD were described such as development of glycosuria by the age of 9.3 weeks. They showed that 72% of surviving R6/2 mice tested positive for glycosuria by 14 weeks of age. In this study, they found that R6/2 mice displayed progressively worsening glucose intolerance. It is perplexing that there was no correlation between the ages of onset of glucosuria and the age at death of R6/2 mice and R6/2 mice with glycosuria die at similar age as those R6/2 mice without it. They tested glibenclamide and rosiglitazone in an acute treatment paradigm in R6/2 mice at 6 weeks and 10 weeks of age. Glibenclamide significantly reduced blood glucose concentrations in R6/2 mice and wild type mice just one hour after challenge, while rosiglitazone did not alter postchallenge blood glucose values in older R6/2 mice or wild type mice. However, the chronic daily treatment with rosiglitazone in combination with glibenclamide significantly reduced the fasting blood glucose concentration in all mice at 10 weeks of age [61]. Although the objective of this study was to examine the onset of diabetes and its possible contribution to the mortality and motor impairment of R6/2 mice, it also provided data for the role of PPAR-γ in R6/2 mice. A recent study also reported the use of metformin, another antidiabetes drug in R6/2 transgenic mice [62]. Metformin treatment in R6/2 mice had beneficial effect only in males with lower doses (2 mg/mL) which translate to about 300 mg/kg/day, and only increased survivals modestly while the fasting daily glucose levels was not changed. Metformin had no effect in females and higher dose (5 mg/mL) had no effect in males' survivals while the glucose level was reduced at 12 weeks of age. Metformin has numerous effects on metabolism, including insulin sensitization [63], increased glucose uptake [64], decrease hepatic glucose synthesis [65], activation of AMP-activated protein kinase (AMPK, an enzyme involved in glucose and fatty acid metabolism) [66], and mitochondrial inhibition [67, 68]. Activation of AMPK is associated with mitochondrial proliferation and biogenesis [69]. Rosiglitazone was used as PPAR-γ agonist in R6/2 mice, which could be used as the bases to test the role of PPAR-γ in HD, glibenclamide and metformin were used to treat atypical diabetes in R6/2 mice. Metformin treatment in R6/2 mice increased brain AMPK phosphorylation [62] although this needs to be confirmed. Activation of AMPK leads to reduction in ATP-consuming processes and facilitate ATP-generating cellular processes which could be an explanation for the metformin effect in R6/2 males. Metformin was also considered to be effective in R6/2 mice because of its ability to sensitize insulin which leads to facilitation of glucose utilization. However, role of metformin in mitochondrial biogenesis in R6/2 mice was not examined. The protective effect of metformin in R6/2 mice could be the synergistic effect from several pathways including regulation of PGC-1α activity through its direct activation of AMPA kinase. Although the exact mechanism underlying mitochondrial biogenesis may vary between tissues, emerging data indicate that substantial overlap exists. Metformin does not belong to any class of PPAR-γ agonists although it is an antidiabetic for type-2 diabetes and stabilizes the glucose level.

PGC-1α has been implicated in mitochondrial biogenesis through its ability to control number of genes such as nuclear respiratory factor-1,-2 (NRF-1,-2), estrogen related receptor α (ERRα), and mitochondrial transcription factor A (Tfam) [70]. Compounds like resveratrol have been implicated in mitochondrial biogenesis [71]. Resveratrol has been shown to activate sirtuin 1 (SIRT1) and results in PPAR-γ-mediated transcriptional repression, inhibition of adipogenesis, enhanced lipolysis, and the release of free fatty acids [72]. Activated SIRT1 leads to deactylation of PGC-1α resulting in an activation of PGC-1α [73]. By deacetylating PGC-1α, SIRT1 represses glycolysis, increase hepatic glucose output, and modulates mitochondrial function and biogenesis [73].

PGC-1α is known as master regulator of mitochondrial biogenesis and is shown to modulate a number of metabolically relevant transcription factors that collectively help in mitochondrial biogenesis (for review see [61, 74]). Although PPAR-γ agonist treatments in R6/2 failed, it is premature to conclude that there is no role for PPARs in HD. Therefore, further studies in other models of HD are required to examine other PPAR-γ agonists. Moreover, the effect of PPAR-γ agonists on the expression and activation of PGC-1α in cell culture models of HD may provide preliminary data to plan full-scale studies in animal models of HD. The rationale for that is based on the increasing evidence that PGC-1α expression which is downregulated in patients with Huntington's disease and in several animal models of this neurodegenerative disorder [70].

Thiazolidiones and rexinoids induce PGC-1α gene transcription in brown and white adipocytes [75]. Since PCG-1α shown to have roles in gluconeogenesis, fatty acid oxidation, and adaptive thermoregulation, then it can be predicted that PPAR-γ agonists could help HD mice to maintain thermoregulatory function when exposed to cold. Based on the studies on PGC-1α knockout mice that shown to have neurodegenerative lesions, particularly in striatum, suggest that PGC-1α may have an important function in neurons [76]. However, the neurodegenerative lesions in PGC-1α knockout mice do not mimic lesions in HD. The role of PPAR-γ in ALS, AD, and Parkinson's disease are backed with evidence [19, 20, 38, 39] while the role of PPAR-γ in HD lacks critical evidence and needs to be studied further. Future studies in other transgenic mouse model of HD could shed light on the role of PPARs in HD. Considering recent results on thermoregulation and mitochondrial biogenesis impairment in HD, and potential neuroprotective role of PGC-1α in HD, PPAR-γ desperately seeking further attention and these types of studies could provide essential data on the role of PPAR-γ in HD.

It is possible that TZDs are also involved in mitochondrial biogenesis [77, 78]. Studies in patients treated with PPAR-γ agonists indicate that the reduction of insulin resistance is resulted from the activation of PPAR-γ [78].

PPAR-γ's natural coactivator is PGC-1α. TZDs can mimic the effect of PGC-1α on PPAR-γ. If PGC-1α levels reduces or become inactivated by acetylation, then the activity of PPAR-γ could be affected.

6. FUTURE PERSPECTIVES

In this review, we highlighted the role of PPAR-γ in neurodegenerative diseases, in particular in a mouse model of ALS and HD. The utilization of pioglitazone in a mouse model of ALS by two independent studies provides strong indication for the involvement of PPAR-γ in ALS. Whether PPAR-γ is involved in HD remains to be clarified as one study showed the treatment of R6/2 mice with rosiglitazone, another PPAR-γ agonist, had no beneficial effect.

In the future, we will explore the mechanisms by which PPAR-γ agonists produce neuroprotection in a mouse model of ALS and test whether PPAR-γ has a role in HD. It would be of great interest to determine whether the effect of PPAR-γ is powered by glial or neuronal cells or both in these models. It would also be of great interest to determine the effect of PPAR-γ agonist on muscles in ALS and HD mouse models. These studies in complement with in vitro cell culture studies are necessary in determining the role of PPAR-γ in ALS and HD. Since a thermoregulatory defect exists in HD mouse models (for review see [70]), it would be very informative to test the effect of PPAR-γ agonists on HD mouse models for their effect in thermoregulation. The activation of PGC-1α in HD mouse models or overexpression of PGC-1α in HD mouse models show efficacy in blockage of neuronal death, and lead to improvement in behavioral phenotypes and increase in survival in several HD mouse models. If these are confirmed, then there is bonafide evidence that activation of PGC-1α could be a great therapeutic strategy for HD. The lack of report on the role of PGC-1α in ALS is a limiting step on the hypothesis that PGC-1α could be a target of investigation or therapeutic for ALS. Mitochondria have been implicated in ALS and PGC-1α has possible role in mitochondrial biogenesis, therefore, it would be informative to examine mitochondrial abnormalities and PGC-1α in ALS. However, since PPAR-γ agonist shown to activate PGC-1α, therefore, there is an indirect possibility that PGC-1α in connection with PPAR-γ could play some role in ALS.

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REFERENCES

[1] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an adipocyte enhancer," *Genes & Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[2] Y. Zhu, C. Qi, J. R. Korenberg, et al., "Structural organization of mouse peroxisome proliferator-activated receptor γ (mPPARγ) gene: alternative promoter use and different splicing yield two mPPARγ isoforms," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 17, pp. 7921–7925, 1995.

[3] C. Blanquart, O. Barbier, J. C. Fruchart, B. Staels, and C. Glineur, "Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 85, no. 2–5, pp. 267–273, 2003.

[4] A. C. Li and C. K. Glass, "PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis," *Journal of Lipid Research*, vol. 45, no. 12, pp. 2161–2173, 2004.

[5] G. Pascual and C. K. Glass, "Nuclear receptors versus inflammation: mechanisms of transrepression," *Trends in Endocrinology & Metabolism*, vol. 17, no. 8, pp. 321–327, 2006.

[6] T. Kielian and P. D. Drew, "Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation," *Journal of Neuroscience Research*, vol. 71, no. 3, pp. 315–325, 2003.

[7] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[8] J. K. Reddy and R. Chu, "Peroxisome proliferator-induced pleiotropic responses: pursuit of a phenomenon," *Annals of the New York Academy of Sciences*, vol. 804, pp. 176–201, 1996.

[9] D. J. Mangelsdorf, C. Thummel, M. Beato, et al., "The nuclear receptor superfamily: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[10] C. Dreyer, G. Krey, H. Keller, F. Givel, G. Helftenbein, and W. Wahli, "Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors," *Cell*, vol. 68, no. 5, pp. 879–887, 1992.

[11] S. A. Kliewer, B. M. Forman, B. Blumberg, et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

[12] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[13] S. Jain, S. Pulikuri, Y. Zhu, et al., "Differential expression of the peroxisome proliferator-activated receptor γ (PPARγ) and its coactivators steroid receptor coactivator-1 and PPAR-binding protein PBP in the brown fat, urinary bladder, colon, and breast of the mouse," *American Journal of Pathology*, vol. 153, no. 2, pp. 349–354, 1998.

[14] S. S.-T. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the α isoform of the peroxisome proliferator- activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, no. 6, pp. 3012–3022, 1995.

[15] K. Tureyen, R. Kapadia, K. K. Bowen, et al., "Peroxisome proliferator-activated receptor-γ agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents," *Journal of Neurochemistry*, vol. 101, no. 1, pp. 41–56, 2007.

[16] S.-W. Park, J.-H. Yi, G. Miranpuri, et al., "Thiazolidinedione class of peroxisome proliferator-activated receptor γ agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1002–1012, 2007.

[17] C. K. Glass and S. Ogawa, "Combinatorial roles of nuclear receptors in inflammation and immunity," *Nature Reviews Immunology*, vol. 6, no. 1, pp. 44–55, 2006.

[18] T. Breidert, J. Callebert, M. T. Heneka, G. Landreth, J. M. Launay, and E. C. Hirsch, "Protective action of the peroxisome proliferator-activated receptor-γ agonist pioglitazone in a mouse model of Parkinson's disease," *Journal of Neurochemistry*, vol. 82, no. 3, pp. 615–624, 2002.

[19] T. Dehmer, M. T. Heneka, M. Sastre, J. Dichgans, and J. B. Schulz, "Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with IκBα induction and block of NFκB and iNOS activation," *Journal of Neurochemistry*, vol. 88, no. 2, pp. 494–501, 2004.

[20] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of β- amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists," *Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[21] J. Auwerx, T.-A. Cock, and C. Knouff, "PPAR-γ: a thrifty transcription factor," *Nuclear Receptor Signaling*, vol. 1, e006, pp. 1–3, 2003.

[22] I. Tzameli, H. Fang, M. Ollero, et al., "Regulated production of a peroxisome proliferator-activated receptor-γ ligand during an early phase of adipocyte differentiation in 3T3-L1 adipocytes," *Journal of Biological Chemistry*, vol. 279, no. 34, pp. 36093–36102, 2004.

[23] T. Shimazu, I. Inoue, N. Araki, et al., "A peroxisome proliferator-activated receptor-γ agonist reduces infarct size in transient but not in permanent ischemia," *Stroke*, vol. 36, no. 2, pp. 353–359, 2005.

[24] S. Sundararajan, J. L. Gamboa, N. A. Victor, E. W. Wanderi, W. D. Lust, and G. E. Landreth, "Peroxisome proliferator-activated receptor-γ ligands reduce inflammation and infarction size in transient focal ischemia," *Neuroscience*, vol. 130, no. 3, pp. 685–696, 2005.

[25] X. Zhao, Y. Zhang, R. Strong, J. C. Grotta, and J. Aronowski, "15d-Prostaglandin J_2 activates peroxisome proliferator-activated receptor-γ, promotes expression of catalase, and reduces inflammation, behavioral dysfunction, and neuronal loss after intracerebral hemorrhage in rats," *Journal of Cerebral Blood Flow & Metabolism*, vol. 26, no. 6, pp. 811–820, 2006.

[26] M. Li, G. Pascual, and C. K. Glass, "Peroxisome proliferator-activated receptor γ-dependent repression of the inducible nitric oxide synthase gene," *Molecular and Cellular Biology*, vol. 20, no. 13, pp. 4699–4707, 2000.

[27] C. G. Su, X. Wen, S. T. Bailey, et al., "A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response," *Journal of Clinical Investigation*, vol. 104, no. 4, pp. 383–389, 1999.

[28] M. Kiaei, K. Kipiani, N. Y. Calingasan, et al., "Matrix metalloproteinase-9 regulates TNF-α and FasL expression in neuronal, glial cells and its absence extends life in a transgenic mouse model of amyotrophic lateral sclerosis," *Experimental Neurology*, vol. 205, no. 1, pp. 74–81, 2007.

[29] M. Kiaei, K. Kipiani, S. Petri, et al., "Integrative role of $cPLA_2$ with COX-2 and the effect of non-steriodal anti-inflammatory drugs in a transgenic mouse model of amyotrophic lateral sclerosis," *Journal of Neurochemistry*, vol. 93, no. 2, pp. 403–411, 2005.

[30] M. Kiaei, S. Petri, K. Kipiani, et al., "Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis," *Journal of Neuroscience*, vol. 26, no. 9, pp. 2467–2473, 2006.

[31] J. S. Welch, M. Ricote, T. E. Akiyama, F. J. Gonzalez, and C. K. Glass, "PPARγ and PPARδ negatively regulate specific subsets of lipopolysaccharide and IFN-γ target genes in macrophages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 11, pp. 6712–6717, 2003.

[32] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[33] D. B. Drachman, K. Frank, M. Dykes-Hoberg, et al., "Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS," *Annals of Neurology*, vol. 52, no. 6, pp. 771–778, 2002.

[34] G. Almer, S. Vukosavic, N. Romero, and S. Przedborski, "Inducible nitric oxide synthase up-regulation in a transgenic mouse model of familial amyotrophic lateral sclerosis," *Journal of Neurochemistry*, vol. 72, no. 6, pp. 2415–2425, 1999.

[35] J. Xu and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia," *Journal of Immunology*, vol. 178, no. 3, pp. 1904–1913, 2007.

[36] M. T. Heneka, D. L. Feinstein, E. Galea, M. Gleichmann, U. Wüllner, and T. Klockgether, "Peroxisome proliferator-activated receptor γ agonists protect cerebellar granule cells from cytokine-induced apoptotic cell death by inhibition of inducible nitric oxide synthase," *Journal of Neuroimmunology*, vol. 100, no. 1-2, pp. 156–168, 1999.

[37] M. T. Heneka, T. Klockgether, and D. L. Feinstein, "Peroxisome proliferator-activated receptor-γ ligands reduce neuronal inducible nitric oxide synthase expression and cell death in vivo," *Journal of Neuroscience*, vol. 20, no. 18, pp. 6862–6867, 2000.

[38] M. Kiaei, K. Kipiani, J. Chen, N. Y. Calingasan, and M. F. Beal, "Peroxisome proliferator-activated receptor-γ agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis," *Experimental Neurology*, vol. 191, no. 2, pp. 331–336, 2005.

[39] B. Schütz, J. Reimann, L. Dumitrescu-Ozimek, et al., "The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice," *Journal of Neuroscience*, vol. 25, no. 34, pp. 7805–7812, 2005.

[40] E. J. Park, S. Y. Park, E.-H. Joe, and I. Jou, "15d-PGJ_2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia," *Journal of Biological Chemistry*, vol. 278, no. 17, pp. 14747–14752, 2003.

[41] P. D. Drew, J. Xu, P. D. Storer, J. A. Chavis, and M. K. Racke, "Peroxisome proliferator-activated receptor agonist regulation of glial activation: relevance to CNS inflammatory disorders," *Neurochemistry International*, vol. 49, no. 2, pp. 183–189, 2006.

[42] M. F. Beal, "Mitochondria take center stage in aging and neurodegeneration," *Annals of Neurology*, vol. 58, no. 4, pp. 495–505, 2005.

[43] S. Sasaki and M. Iwata, "Impairment of fast axonal transport in the proximal axons of anterior horn neurons in amyotrophic lateral sclerosis," *Neurology*, vol. 47, no. 2, pp. 535–540, 1996.

[44] M. T. Lin and M. F. Beal, "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," *Nature*, vol. 443, no. 7113, pp. 787–795, 2006.

[45] J. Kong and Z. Xu, "Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral

sclerosis in mice expressing a mutant SOD1," *Journal of Neuroscience*, vol. 18, no. 9, pp. 3241–3250, 1998.

[46] M. Mattiazzi, M. D'Aurelio, C. D. Gajewski, et al., "Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice," *Journal of Biological Chemistry*, vol. 277, no. 33, pp. 29626–29633, 2002.

[47] C. M. J. Higgins, C. Jung, and Z. Xu, "ALS-associated mutant $SOD1^{G93A}$ causes mitochondrial vacuolation by expansion of the intermembrane space by involvement of SODI aggregation and peroxisomes," *BMC Neuroscience*, vol. 4, article 16, pp. 1–14, 2003.

[48] P. Pasinelli and R. H. Brown, "Molecular biology of amyotrophic lateral sclerosis: insights from genetics," *Nature Reviews Neuroscience*, vol. 7, no. 9, pp. 710–723, 2006.

[49] N. C. Myrianthopoulos, "Huntington's chorea," *Journal of Medical Genetics*, vol. 3, no. 4, pp. 298–314, 1966.

[50] Z. Ortega, M. Díaz-Hernández, and J. J. Lucas, "Is the ubiquitin-proteasome system impaired in Huntington's disease?" *Cellular and Molecular Life Sciences*, vol. 64, no. 17, pp. 2245–2257, 2007.

[51] J. Arenas, Y. Campos, R. Ribacoba, et al., "Complex I defect in muscle from patients with Huntington's disease," *Annals of Neurology*, vol. 43, no. 3, pp. 397–400, 1998.

[52] W. A. Brennan Jr., E. D. Bird, and J. R. Aprille, "Regional mitochondrial respiratory activity in Huntington's disease brain," *Journal of Neurochemistry*, vol. 44, no. 6, pp. 1948–1950, 1985.

[53] S. E. Browne, A. C. Bowling, U. Macgarvey, et al., "Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia," *Annals of Neurology*, vol. 41, no. 5, pp. 646–653, 1997.

[54] M. Gu, M. T. Gash, V. M. Mann, F. Javoy-Agid, J. M. Cooper, and A. H. V. Schapira, "Mitochondrial defect in Huntington's disease caudate nucleus," *Annals of Neurology*, vol. 39, no. 3, pp. 385–389, 1996.

[55] W. D. Parker Jr., S. J. Boyson, A. S. Luder, and J. K. Parks, "Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease," *Neurology*, vol. 40, no. 8, pp. 1231–1234, 1990.

[56] L. Cui, H. Jeong, F. Borovecki, C. N. Parkhurst, N. Tanese, and D. Krainc, "Transcriptional repression of PGC-1α by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration," *Cell*, vol. 127, no. 1, pp. 59–69, 2006.

[57] J. St-Pierre, S. Drori, M. Uldry, et al., "Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators," *Cell*, vol. 127, no. 2, pp. 397–408, 2006.

[58] P. Weydt, V. V. Pineda, A. E. Torrence, et al., "Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1α in Huntington's disease neurodegeneration," *Cell Metabolism*, vol. 4, no. 5, pp. 349–362, 2006.

[59] A. W. Deckel, "Nitric oxide and nitric oxide synthase in Huntington's disease," *Journal of Neuroscience Research*, vol. 64, no. 2, pp. 99–107, 2001.

[60] S. E. Browne, R. J. Ferrante, and M. F. Beal, "Oxidative stress in Huntington's disease," *Brain Pathology*, vol. 9, no. 1, pp. 147–163, 1999.

[61] M. J. Hunt and A. J. Morton, "Atypical diabetes associated with inclusion formation in the R6/2 mouse model of Huntington's disease is not improved by treatment with hypoglycaemic agents," *Experimental Brain Research*, vol. 166, no. 2, pp. 220–229, 2005.

[62] T. C. Ma, J. L. Buescher, B. Oatis, et al., "Metformin therapy in a transgenic mouse model of Huntington's disease," *Neuroscience Letters*, vol. 411, no. 2, pp. 98–103, 2007.

[63] E. I. Widen, J. G. Eriksson, and L. C. Groop, "Metformin normalizes nonoxidative glucose metabolism in insulin-resistant normoglycemic first-degree relatives of patients with NIDDM," *Diabetes*, vol. 41, no. 3, pp. 354–358, 1992.

[64] D. Galuska, J. Zierath, A. Thörne, T. Sonnenfeld, and H. Wallberg-Henriksson, "Metformin increases insulin-stimulated glucose transport in insulin-resistant human skeletal muscle," *Diabete et Metabolisme*, vol. 17, no. 1, pp. 159–163, 1991.

[65] R. S. Hundal, M. Krssak, S. Dufour, et al., "Mechanism by which metformin reduces glucose production in type 2 diabetes," *Diabetes*, vol. 49, no. 12, pp. 2063–2069, 2000.

[66] G. Zhou, R. Myers, Y. Li, et al., "Role of AMP-activated protein kinase in mechanism of metformin action," *Journal of Clinical Investigation*, vol. 108, no. 8, pp. 1167–1174, 2001.

[67] D. Detaille, B. Guigas, X. Leverve, N. Wiernsperger, and P. Devos, "Obligatory role of membrane events in the regulatory effect of metformin on the respiratory chain function," *Biochemical Pharmacology*, vol. 63, no. 7, pp. 1259–1272, 2002.

[68] M. R. Owen, E. Doran, and A. P. Halestrap, "Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain," *Biochemical Journal*, vol. 348, part 3, pp. 607–614, 2000.

[69] B. Chabi, P. J. Adhihetty, V. Ljubicic, and D. A. Hood, "How is mitochondrial biogenesis affected in mitochondrial disease?" *Medicine & Science in Sports & Exercise*, vol. 37, no. 12, pp. 2102–2110, 2005.

[70] J. K. McGill and M. F. Beal, "PGC-1α, a new therapeutic target in Huntington's disease?" *Cell*, vol. 127, no. 3, pp. 465–468, 2006.

[71] Y. Kraytsberg, E. Kudryavtseva, A. C. McKee, C. Geula, N. W. Kowall, and K. Khrapko, "Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons," *Nature Genetics*, vol. 38, no. 5, pp. 518–520, 2006.

[72] F. Picard, M. Kurtev, N. Chung, et al., "Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ," *Nature*, vol. 429, no. 6993, pp. 771–776, 2004.

[73] S. Michan and D. Sinclair, "Sirtuins in mammals: insights into their biological function," *Biochemical Journal*, vol. 404, no. 1, pp. 1–13, 2007.

[74] S. M. Houten and J. Auwerx, "PGC-1α: turbocharging mitochondria," *Cell*, vol. 119, no. 1, pp. 5–7, 2004.

[75] E. Hondares, O. Mora, P. Yubero, et al., "Thiazolidinediones and rexinoids induce peroxisome proliferator-activated receptor-coactivator (PGC)-1α gene transcription: an autoregulatory loop controls PGC-1α expression in adipocytes via peroxisome proliferator-activated receptor-γ coactivation," *Endocrinology*, vol. 147, no. 6, pp. 2829–2838, 2006.

[76] J. Lin, P.-H. Wu, P. T. Tarr, et al., "Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice," *Cell*, vol. 119, no. 1, pp. 121–135, 2004.

[77] S. Ghosh, N. Patel, D. Rahn, et al., "The thiazolidinedione pioglitazone alters mitochondrial function in human neuron-like cells," *Molecular Pharmacology*, vol. 71, no. 6, pp. 1695–1702, 2007.

[78] R. H. Swerdlow, "Treating neurodegeneration by modifying mitochondria: potential solutions to a "complex" problem," *Antioxidants & Redox Signaling*, vol. 9, no. 10, pp. 1591–1603, 2007.

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Review Article

Regulation of Glial Cell Functions by PPAR-γ Natural and Synthetic Agonists

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In the recent years, the peroxisome proliferator-activated receptor-γ (PPAR-γ), a well known target for type II diabetes treatment, has received an increasing attention for its therapeutic potential in inflammatory and degenerative brain disorders. PPAR-γ agonists, which include naturally occurring compounds (such as long chain fatty acids and the cyclopentenone prostaglandin 15-deoxy $\Delta^{12,14}$ prostaglandin J_2), and synthetic agonists (among which the thiazolidinediones and few nonsteroidal anti-inflammatory drugs) have shown anti-inflammatory and protective effects in several experimental models of Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, multiple sclerosis and stroke, as well as in few clinical studies. The pleiotropic effects of PPAR-γ agonists are likely to be mediated by several mechanisms involving anti-inflammatory activities on peripheral immune cells (macrophages and lymphocytes), as well as direct effects on neural cells including cerebral vascular endothelial cells, neurons, and glia. In the present article, we will review the recent findings supporting a major role for PPAR-γ agonists in controlling neuroinflammation and neurodegeneration through their activities on glial cells, with a particular emphasis on microglial cells as major macrophage population of the brain parenchyma and main actors in brain inflammation.

1. INTRODUCTION

The peroxisome proliferator-activated receptor-γ (PPAR-γ) belongs to the hormone nuclear receptor super family. It is a ligand-dependent transcription factor activated by both naturally occurring compounds, such as long chain fatty acids and the cyclopentenone prostaglandin 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 (15d-PGJ_2), and synthetic agonists, including the thiazolidinediones (TZDs), and few nonsteroidal anti-inflammatory drugs (NSAIDs). Because of their role in the regulation of genes involved in lipid and carbohydrate metabolism, PPAR-γ and the other two isoforms PPAR-α and δ, deeply affect lipid homeostasis and insulin sensitivity [1–3]. The TZDs rosiglitazone (Avandia®), and pioglitazone (Actos®), introduced on the market in the early 1990s, are currently in clinical use to control blood glucose levels in subjects affected by type II diabetes.

In the last decade, accumulating evidence suggests that, besides diabetes and metabolic syndrome [4], PPAR-γ agonists have significant therapeutic potential in brain disorders. A large number of experimental studies and few clinical observations have suggested that PPAR-γ ligands may be successfully exploited to treat a wide range of neurological diseases, ranging from neurodegenerative diseases, to traumatic injuries, stroke, and demyelinating diseases, as recently reviewed by Heneka et al. [5]. In Alzheimer's disease (AD) transgenic mouse models, the TZD rosiglitazone attenuated learning and memory deficits [6], in line with its ability to promote cognitive preservation in patients with early AD [7, 8]. In amyotrophic lateral sclerosis (ALS) and Parkinson's disease animal models, the TZD pioglitazone ameliorated the disease symptoms [9, 10]. In rodent focal ischemia models, both pioglitazone and rosiglitazone decreased the infarct volume [11–13]. Furthermore, the natural agonist 15d-PGJ_2 was shown to decrease the neurological deficits after experimental intracerebral hemorrhage [14] and its plasma levels in stroke patients were found directly correlated to the neurological outcome [15]. Rosiglitazone and pioglitazone decreased secondary neuronal damage, astrogliosis, microglial activation, myelin loss, and neuropathic pain

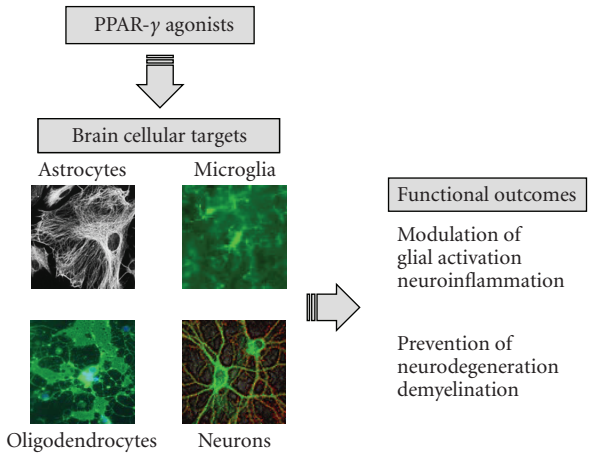

Figure 1: Cellular targets of PPAR-γ agonists in neurodegenerative diseases. PPAR-γ agonists can control neuroinflammation, neurodegeneration, and demyelination by effecting several cellular targets and by several direct and indirect mechanisms. PPAR-γ agonists can control glial activation, preventing a number of proinflammatory activities that can contribute to myelin/OL damage and neurotoxicity PPAR-γ agonists may also affect OLs and neurons, by preventing release inflammatory mediators and/or promote the synthesis of soluble factors or membrane-bound molecules that control glial activation.

in animal models of spinal cord injury while improving motor function recovery [16]. In experimental autoimmune encephalomyelitis (EAE), a well known model for autoimmune demyelinating diseases, synthetic, and natural PPAR-γ ligands—as well as some PPAR-α or δagonists—have been reported to ameliorate clinical symptoms, to reduce expression of pro-inflammatory cytokines and chemokines, to decrease brain inflammation, demyelination and glial activation, and to delay the onset of disease [17–25]. More recently, promising results obtained in experimental models of ocular diseases have evidenced that PPAR-γ could be targeted to control inflammation and treat invalidating diseases such as diabetic retinopathy and optic neuritis, a demyelinating disease of the optic nerve frequently associated to multiple sclerosis (MS) (see for review [26]). Nonetheless, in spite of the amount of data on the therapeutic activities of PPAR agonists in EAE, clinical studies are still lacking and reports on their clinical use in MS or optic neuritis are still anecdotal [27]. Clinical trials are, however, in course with pioglitazone and rosiglitazone [5].

The beneficial effects of PPAR-γ agonists in degenerative, inflammatory and traumatic brain pathologies are most likely mediated by several mechanisms, which may be disease-specific and involve both peripheral and central anti-inflammatory activities, by affecting crucial functions of peripheral (macrophages and/or lymphocytes) and central (microglial cells) immune cells. Besides microglia, PPAR-γ agonists can act on other neural cell types, including astrocytes, neurons, and oligodendrocytes (Figure 1).

Several of the beneficial effects of PPAR-γ result from its ability, once activated by specific ligand, to control the expression of proinflammatory genes, through the binding of specific sequences in their promoter regions—the peroxisome proliferator response elements (PPREs)—but also independently from its DNA-binding activity, by a mechanism termed transrepression, which have just begun to be elucidated [28]. In addition, some PPAR-γ ligands may exert specific activities independently from PPAR-γ. Among these, of great interest is the ability of a few TZDs to directly influence mitochondrial function by binding to target sites in mitochondria including the Complex I of the respiratory chain and the newly described protein mitoneet [29].

2. PPAR-γ: STRUCTURE, FUNCTIONS, AND AGONISTS

The PPAR-γ and the two closely related PPAR-α and PPAR-δ (also known as β, NUC-1, or FAAR) share a high homology, but differ for tissue distribution and ligand specificity [2]. PPAR-α is mainly expressed in tissues with high catabolic rates of fatty acids, such as the liver, muscle, and heart, whereas PPAR-δ shows a much wider distribution. PPAR-γ is highly expressed in adipose tissue and in cells of the immune system, including lymphocytes and macrophages. In the brain, PPAR-γ is expressed in several cell types including microglia, astrocytes, oligodendrocytes, and neurons.

PPAR-γ protein shows a remarkable conservation across species. Human and the murine PPAR-γ proteins show 95% identity at the amino acid level. The human PPAR-γ gene is located on chromosome 3 and generates at least three mRNA transcripts, PPAR-γ1, PPAR-γ2, and PPAR-γ3 [30–32]. PPAR-γ1 e PPAR-γ3 mRNAs encode for the same protein, while PPAR-γ2 mRNA gives rise to a protein containing 28 additional amino acids at the N-terminus.

At protein level, all three PPARs show a similar organization in five different functional domains, two of which—the DNA-binding domain (DBD) and the ligand-binding domain (LBD)—are the highly conserved [2]. The DBD contains the two zinc finger-like motifs that recognize the DNA target, and can be considered the hallmark of the nuclear receptor superfamily. The LBD conserves a common three-dimensional structure, which hosts a particularly large ligand-binding cavity, of which only 30–40% is occupied by the ligand. The relatively free nonspecific interaction between the cavity and the hydrophobic domains of the ligand explains the low ligand-specificity of PPARs. Nonetheless, the LBDs of the three PPAR isotypes have sufficiently divergent amino acid sequences to allow some ligand specificity.

Several unsaturated fatty acids bind to all three PPAR isoforms, whereas saturated fatty acids are in general poor PPAR ligands. However, given the relatively high concentration of lipids required for PPAR activation (in the micromolar or submicromolar concentration range), their "in vivo" role as PPAR ligands remains a controversial issue. Some arachidonic acid metabolites are more effective PPAR-γ ligands than the precursor. In particular, 15d-PGJ_2, characterized by a reactive α,β-unsaturated ketone in the cyclopentenone ring, was the first PPAR-γ endogenous ligand, described in 1995 by two independent groups [33, 34].

The implication of PPAR-γ in several important metabolic and degenerative disorders, has strongly pushed the research of specific PPAR-γ agonists and antagonist (for review see [35]). A major group of synthetic PPAR-γ agonists is represented by the antidiabetic drugs TZDs, originally identified for their ability to improve the insulin sensitivity of diabetic animals. Pioglitazone and rosiglitazone belong to this group of high-affinity ligand. A different series of synthetic PPAR-γ ligands are derived by L-tyrosine GI262570, GW1929, and GW7845, which were developed on the basis of their activity on human PPAR-γ and are among the most potent PPAR-γ agonists, being active at low nanomolar concentrations.

In addition to these groups of ligands, several members of the heterogeneous NSAID family have been described as agonists for PPARs [35] and reference therein. In most cases, the doses required for PPAR-γ agonist activity are in the high micromolar range, thus largely exceeding those required for in vivo inhibition of cyclooxygenases (COXs), the main target of these drugs. Among NSAIDs, aspirin and acetaminophen (or paracetamol) lack of agonistic activity for any of the PPAR subtypes, whereas indomethacin, ibuprofen, and diclofenac are selective for the γsubtype. Recently, we have shown that the two nitric oxide (NO)-releasing derivative of flurbiprofen, HCT1026 and NXC 2216, were both able to activate PPAR-γ and induce its specific binding to a PPRE sequence [36, 37]. Few antagonists are also available, but their use is often limited by partial agonistic activity. The plasticizer biphenol A diglycidyl ether (BADGE) and the irreversible antagonist GW9662 are among the most widely used.

3. PPAR-γ AGONISTS AND OLIGODENDROCYTE BIOLOGY

Oligodendrocytes (OLs) are the myelin-forming cells of the CNS. Their differentiation from precursor to mature cells occurs through a series of stages that can be defined by morphological and antigenic changes occurring in vivo as well as in culture systems [38]. During development and repair OLs extend elongated processes, forming multilamellar sheaths around neuronal axons. The formation, growth, and maintenance of the myelin sheath are prominent parts of neural development and nervous system function. As for OL maturation, myelin formation is a multistep process, involving recruitment to germination sites, proliferation of undifferentiated OL progenitors and their differentiation to mature OLs, producing myelin. Damage to OLs as a result of oxidative stress is considered a key pathogenetic pathway in several adult and infant human diseases. A substantial number of in vitro and in vivo studies has shown a maturation-dependent vulnerability to oxidative stress of the OL lineage [39–41], suggesting that OL progenitor is a key target for limit white matter damage and promote myelin repair [42]. Oligodendrocytes are major lipid producing cells, as required for myelin formation and maintenance. Given the role of PPARs in lipid metabolism it is conceivable that this group of nuclear receptor play a major role in OL differentiation and function. Although PPAR-β/δ has been

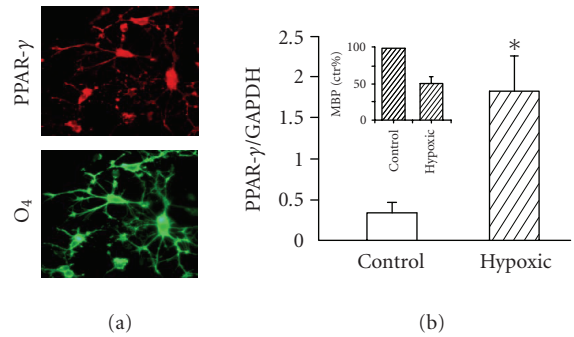

Figure 2: PPAR-γ expression in culture rat oligodendrocytes and in white matter (postnatal day 19) in rat model of global perinatal asphyxia. (a) Immunocytochemistry of rat OL progenitor cultures, prepared as previously described [40] for PPAR-γ (upper panel) and the OL marker O4 (lower panel). (b) Western blot analysis of white matter homogenates from rats at postnatal day 19 subjected to 20 minutes of perinatal asphyxia (hypoxic) and from controls, prepared as described in Piscopo et al. [48]. Inset show the decreased levels of MBP in hypoxic rats at pnd 19.

long considered the PPAR type mainly expressed in OLs and involved in myelination [43, 44], recent findings support an important role for PPAR-γ activators in OL protection and differentiation. The first evidence for a role of PPAR-γ in OL differentiation was reported by Roth et al. [45]. By using the B12, oligodendrocyte-like cell line and primary cultures of spinal cord OL precursors, the authors first demonstrated that these cells expressed all three PPAR isoforms and found that natural and synthetic PPAR-γ agonists, but not other isoform activators, enhance process extension and cell maturation. These effects were blocked by the PPAR-γ antagonist GW9662. The maturation of pre-OLs was accompanied by enhanced expression of alkyl-dihydroxyacetone phosphate synthase (ADAPS), a peroxisomal enzyme required for the synthesis of plasmalogen, an etherphospholipid essential for myelin formation. These observations suggest that PPAR-γ mediated mechanisms may be important for OL differentiation and peroxisome functions. An important role for these organelles in maintaining OL and white matter integrity has been recently demonstrated in mutant mice characterized by the selective absence of functional peroxisomes from OLs [46]. In line with the proposed role of PPAR-γ in controlling OL differentiation and functions, we have recently confirmed the expression of PPAR-γ in highly purified rat OL cultures (Figure 2(a)). The level of expression is increased with the OL maturation in vitro (Bernardo et al., in preparation). In addition, we found an increased expression of PPAR-γ in white matter of young rats (post natal day 19) exposed to perinatal global asphyxia (Figure 2(b)). This model mimics some of the features of perinatal asphyxia, a major cause of immediate and delayed brain damage in the newborn [47, 48], and is characterized by early oxidative stress, delayed behavioral deficits, and alteration in myelin formation, as indicated by the strong reduction of myelin basic protein (MBP) expression (Figure 2(b)). Whether PPAR-γ overexpression is part of an adaptive response to the hypoxic

condition aimed at restoring myelin formation or is part of an aberrant program leading behavioral impairment remain to be established.

In apparent contrast with the above findings, Xiang et al. [49], reported that the PPAR-γ natural ligand 15d-PGJ_2, but not other PGs, induced apoptosis of OL precursor cell lines (mOP and CG4 cell lines). The toxic effect was developmental stage-dependent, being the undifferentiated mOP cells more susceptible than differentiated cells. In line with observations previously reported in microglia cultures [50], cell death was independent of the nuclear receptor PPAR-γ. Since the toxic effect of 15d-PGJ_2 was prevented by preincubation of cell cultures with N-acetyl cysteine, a reducing agent and a precursor molecule for glutathione (GSH) synthesis, but not with free radical scavengers, the authors suggest that the underlying mechanism is related to oxidative stress due to depletion of GSH.

4. PPAR-γ AGONISTS AND ASTROCYTES

Astrocytes are most abundant glial cells in the CNS and crucial players in brain homeostasis. Among other functions, they provide metabolic support for neurons, uptake neurotransmitters such as glutamate, synthesize neurotrophic factors, and contribute to ion homeostasis (i.e., potassium uptake) and blood-brain barrier induction and maintenance [51]. In addition, astrocytes exert important roles also in brain inflammation and immunity, as they express several—though fewer than microglia—pattern-recognition receptors (PRRs) such as for example the Toll-like receptors, and release cytokines and chemokines that can trigger or amplify the local inflammatory response [52]. Similar to microglia, astrocytes rapidly react to a wide array of insults or damaging events. Reactive astrocytes, which are characterized by increased expression of glial fibrillary acidic protein (GFAP), a constituent of the intermediate filaments, are typical of most brain pathologies. Thus astrocytes represent an important target for anti-inflammatory and neuroprotective therapeutic strategies.

Astrocytes express PPAR-γ [53, 54], and accumulating evidence over the last ten years indicates that PPAR-γ agonists modulate astrocyte functions.

In rat cortical slices and cultured astrocytes, the TZD pioglitazone was found to significantly increase glucose consumption in time- and dose-dependent manners, through a mechanism independent of PPAR-γ and involving cAMP/PKA signaling [55]. Pioglitazone did not modify the expression of the glucose transporter GLUT-1, which is mainly expressed in glial and endothelial cells, but rather it increased glucose flux through pre-existing GLUT-1 protein. In addition, pioglitazone increased lactate production and release, induced mitochondrial membrane hyperpolarization, and protected astrocytes against hypoglycemia-induced cell death. On the basis of their studies, the authors suggest that TZDs modulate enzyme activities present within the mitochondrial membrane causing increased cytosolic pyruvate, resulting in greater lactate production. The inhibitory effect on mitochondrial function is compensated by an increase in anaerobic glycolysis allowing for continued ATP production. Eventually, the reduced intracellular glucose levels are replenished by glucose transport through the GLUT-1. At later times, mitochondrial respiration recovers, and accumulated ATP utilized to maintain and increase the membrane potential. Because hyperpolarization of the mitochondrial membrane is postulated to be protective, the net result of TZD treatment, at least in astrocytes, is protective and allows cells to withstand subsequent noxious stimuli [55]. Altogether, these results suggest that TZD-induced alteration of astrocyte metabolism and mitochondrial function could be beneficial in neurological conditions, in which glucose availability is reduced.

Another important mechanism by which PPAR-γ agonists could exert neuroprotection by influencing astrocyte functions is the enhancement of glutamate uptake. Romera et al. [56] reported that the PPAR-γ antagonists T0070907 prevented the ischemic preconditioning-induced (IPC) neuroprotection in neuronal-astrocytic cocultures subjected to oxygen-glucose deprivation (ODG) and reversed the inhibitory effect of IPC on OGD-induced glutamate release. In addition, rosiglitazone and the non-TZD agonist L-796,449 induced a concentration-dependent increase in glutamate transporter GLT-1 expression and [^{3}H] glutamate uptake in rat astrocytes. In addition the authors identified six putative PPREs in the promoter region of GLT1/EAAT2 gene, suggesting GLT1/EAAT2 glutamate transporter is a novel PPAR-γ target gene [56]. Finally, 15d-PGJ_2 remarkably increase the synthesis and release of neurotrophic factor nerve growth factor (NGF) in mouse primary astrocytes, which could further contribute to neuroprotection [57].

As mentioned above, activated astrocytes produce cytokines and other molecules involved in inflammatory response, which are thought to significantly contribute to brain damage. Such neurotoxic activities have been shown to be reduced by PPAR-γ agonists in several experimental paradigms. The two TZD compounds NP00111 and NP01138 were reported to inhibit the production of nitric oxide (NO), IL-6, and TNF-α as well as expression of the inducible enzymes iNOS and COX2 induced in LPS-stimulated astrocyte and microglial cultures [58]. Consistently with the described anti-inflammatory activities, the two compounds were neuroprotective in an animal model in which of brain damage is induced by kainic acid administration [59]. Both in vitro and in vivo effects were substantially attenuated by cotreatment with the PPAR-γ antagonist GW9662, supporting the involvement of PPAR-γ activation.

In contrast to the above described TZDs, the natural ligand 15d-PGJ_2 prevented the IL-1β-induced COX-2 mRNA accumulation in human astrocytes, through a PPARγ-independent mechanism [60]. Similarly, Lennon and colleagues [61] showed that ciglitazone and 15d-PGJ_2 activated the MAP kinase cascades (Erk, Jnk, and p38 MAP kinase) in astrocytes by a PPAR-γ independent mechanism, which required the presence of ROS. Again, independently of PPAR-γ, 15d-PGJ_2 and rosiglitazone reduced the phosphorylation of signal transducers and activators of transcription (STAT) 1 and 3 as well as Janus kinase 1 (JAK1) and JAK2 in activated astrocytes and microglia [62].

Recently, Xu and Drew [63] extended the analysis of the anti-inflammatory activity of PPAR-ligands to other inflammatory mediators belonging to the IL-12 family of cytokines. They found that in primary astrocytes, LPS induced the production of IL-12p40, IL-23, and IL-27p28 proteins, which was significantly reduced in the presence of 15d-PGJ_2. Since these cytokines play critical roles in the differentiation of T helper (Th) 1 and Th17 cells and are likely to contribute to the development of multiple sclerosis, this observation further support the potential role of PPAR-γ agonists in MS treatment [5, 64].

In line with the beneficial effect of PPAR-γ agonists in experimental models of inflammatory diseases, PPAR-γ has also been involved in anti-inflammatory functions of IL-4, a Th2 type cytokine, which plays an important role in controlling Th1 cell responses and resolution of inflammation. Paintlia et al. [65] demonstrated that the inhibition of iNOS expression and the increase of survival of differentiating OPs induced by IL-4 in inflammatory cytokine-stimulated mixed cultures are mediated by PPAR-γ activation. In support of their conclusions, the authors describe a coordinate increase in the expression of both PPAR-$\gamma$and its natural ligand-producing enzyme 12/15-lipoxygenase (12/15-LOX) in IL-4-treated glial cells and show that IL-4-induced PPAR-γ activation antagonizes NF-κB transactivation in inflammatory cytokine-stimulated astrocytes. A similar upregulation of PPAR-γ by IL-4 was demonstrated in cultured microglial cells [66]. To link between IL-4 and PPAR-γ is completed by the observation that the anti-inflammatory activity of the TZD troglitazone was mediated by its ability to increase IL-4 expression in glial cultures [67].

Astrocytes recognize and react to several pathogens through their repertoire of PPRs [52]. In a recent study, 15d-PGJ_2 and ciglitazone suppress the production of IL-1β and NO in Staphylococcus aureus-stimulated astrocytes [68]. Interestingly, 15d-PGJ_2 attenuated TLR2 expression, the PPR recognizing Staphylococcus aureus. Importantly, 15d-PGJ_2 and ciglitazone were still capable of inhibiting the release of proinflammatory mediators induced by Staphylococcus aureus in PPAR-γ-deficient astrocytes, supporting PPAR-γ-independent effects of these compounds. In another study, 15d-PGJ_2 significantly attenuated astrocyte reaction to mycotoxin ochratoxin A (OTA), a widespread food contaminant that accumulates in the brain. At noncytotoxic concentrations, OTA down-regulated GFAP expression while it upregulated vimentin. Interestingly, OTA increased PPAR-γ expression, possibly increasing the susceptibility of OTA-exposed cells to PPAR-γ agonist treatment [69].

5. PPAR-γ AGONISTS AND MICROGLIAL CELLS

Microglia derive from myeloid precursors that enter the developing CNS to become the major population of brain resident macrophages. Under physiological conditions, microglia show a ramified morphology and the absence of cell-surface and cytoplasmic molecules typically associated with other tissue macrophages. In this quiescent or "resting" state microglia are able to "sense" subtle environmental changes to which they rapidly react [70]. Although our knowledge on microglial in physiological conditions is still limited, using transgenic mice showing specific expression of enhanced green fluorescent protein in microglia and in vivo two-photon microscopy, it was shown that "resting" microglia constantly survey the surrounding microenvironment with extremely motile processes and protrusions [71]. Once activated, microglia rapidly undergo morphological changes, characterized by cell body enlargement, loss of ramified processes, and upregulation of cell-surface and/or cytoplasmic antigens. In addition, activated microglia can synthesize a range of different molecules, including free radicals, inflammatory cytokines, chemokines, lipid mediators, and neurotrophic factors, whose typical profile will determine the outcome of microglial activation in term of repair or injury [70]. Although in the past activated microglia have been regarded mainly as detrimental for the surrounding cells and as major players in neurodegenerative processes, it is now clear that activated microglia play complex and multifaceted roles, which need to be defined within each disease. Importantly, the different states of activation can be switched between one state and another during the course of disease or in response to further stimuli or signals from the periphery [72].

A deeper knowledge of microglial biology and of the molecular mechanisms underlying the acquisition of protective versus detrimental functions is crucial for finding new molecular targets and developing effective treatments for a wide range of neurological disorders.

In this view, PPAR-γ agonists have been extensively studied in the last decade for their therapeutic potential as key molecules in preventing the undesired toxic effects of microglial activation [35, 73].

One of the first finding supporting a role for 15d-PGJ_2 as endogenous regulator of microglial activation—15d-PGJ_2 derives from PGD_2, a major PG synthesized within the brain by most neural cells—was provided by Petrova et al. [74], who demonstrated that this PPAR-γ natural ligand attenuates iNOS expression, and the subsequent NO accumulation, in the murine BV-2 microglial cell line stimulated by LPS. Since the TZD troglitazone did not affect the NO pathway, it was suggested that 15d-PGJ_2 inhibits iNOS expression by a PPAR-γ independent mechanism. The same authors then demonstrated that 15d-PGJ_2 decreases the production of TNF-α, IL-1β and the expression of COX-2 in the same cell system while increasing the expression of the antioxidant enzyme hemeoxygenase-1 and the intracellular levels of glutathione [75].

Bernardo et al. [76] showed for the first time that primary microglial cells, unlike BV-2 cells, express PPAR-γ and that such basal expression is increased by its specific agonists, while it is reduced in the presence of microglial activators such as LPS and IFN-γ. Microglial PPAR-γ was subsequently shown to be functionally active, being able to bind specific PPRE sequences upon activation by natural and synthetic agonists [50]. Similar to BV-2 cell line, in primary microglial cultures 15d-PGJ_2 prevented LPS-induced iNOS expression and TNF-α production as well as IFN-γ-induced expression of major histocompatibility complex (MHC) class II antigens, by mechanisms involving

PPAR-γ activation and reduced activation of STAT-1 and NF-κB, which are known to mediate IFN-γ and LPS signaling [76]. In human microglial cells, 15d-PGJ_2 did not affect NF-κB binding activity although it decreased STAT-1 expression and enhanced expression and binding activity of the AP-1 proteins J-Jun and c-Fos [60]. It was then reported that 15d-PGJ_2 inhibits IL-12 synthesis in rat primary microglia and mouse cell line N9, activated either by LPS alone or in combination with IFN-γ or TNF-α [63, 77]. 15d-PGJ_2 attenuated microglial activation also when elicited by Gram-positive bacteria Staphylococcus aureus, by inhibiting the expression of proinflammatory cytokines and the chemokine monocyte chemoattractant protein-1 (MCP-1) [73, 78].

In cortical mixed neuron-glial cultures 15d-PGJ_2, ciglitazone and troglitazone prevented LPS-induced neuronal death, suggesting a PPAR-γ mediated mechanism of neuroprotection [79]. Similarly, 15d-PGJ_2, ciglitazone, troglitazone and two NSAIDs indomethacin and ibuprofen reduced the neurotoxicity of microglial cells exposed to β-amyloid fibrils [80]. In this cell system, COX-2-specific inhibitors failed to promote neuronal survival, suggesting protective mechanisms independent of COX-2 metabolism.

In addition to indomethacin and ibuprofen, we have reported that two NO-releasing derivative of flurbiprofen, HCT1026 and NXC 2216, were able to prevent microglial activation by activating PPAR-γ [36, 37]. Interestingly, NCX 2216 after an initial activation induced PPAR-γ nitration and inactivation. These effects were paralleled by a transient reduction of TNF-α and NO production and a protracted inhibition of IL-1β and PGE_2 synthesis, suggesting a dynamic regulation of the functional state of activated microglia by NCX 2216. Long treatment with NCX 2216 could therefore lead, after an initial activation of PPAR-γ, to a protracted suppression of its control over microglial activation. Our results could help explaining why among the few NSAIDs with Aβ-lowering activity (reviewed by [81]), only in the case of protracted administration of NCX 2216 in an AD animal model, the reduction of cerebral amyloid load accompanied by a sustained microglial activation [82].

The contribution of PPAR-γ-dependent or independent mechanisms to the inhibition of microglial activation by 15d-PGJ_2 seems dependent on the cell type (primary versus transformed cell lines; fetal versus neonatal), or on concentration of the ligand. In rat primary microglial cultures, we have shown [50] that 15d-PGJ_2 at concentrations several fold lower than those required for PPAR-γ activation, effectively reduced the LPS-stimulated production of PGE_2 by directly preventing the enzymatic activity of COX-2 rather than its expression, as previously described in activated monocytic cell lines [80, 83] and in BV-2 cells [75]. The reduction of COX-2 enzymatic activity could be achieved through the modifications of key cysteine residues [84], as suggested by the ability of 15d-PGJ_2 electrophilic α,β-unsaturated ketones to modify several cellular proteins [85, 86]. At concentration 10 times higher than those required to activate PPAR-γ, 15d-PGJ_2 induced microglial cell impairment and death by apoptosis [50]. The effects were stronger in activated microglia than in unstimulated cells, suggesting that this agent may prevent excessive microglial activation by promoting their elimination by apoptosis thus contributing to the resolution of inflammation as previously suggested in peripheral tissues [87, 88].

Although apoptosis by 15d-PGJ_2 has been shown in several cells, the link between the proapoptotic effect of 15d-PGJ_2 and PPAR-γ activation is still controversial. As before this may be linked to cell types and their degree of differentiation or transformation. For example, as opposed to the observations reported in primary microglia, the induction of apoptosis in T-cells and human and rat glioma cell lines appears mediated by PPAR-γ-dependent mechanisms [61, 87, 89–91].

6. CONCLUSIONS

In the last decade, there has been an increasing number of experimental studies supporting the use of PPAR-γ ligands to treat major disabling brain diseases, with a high social burden and impact on health case system. The compelling evidence obtained in experimental studies is complemented by sparse, but very encouraging clinical studies. The positive outcomes in animal models of AD, due to the ability of PPAR-γ agonists to reduced inflammation and the amyloid burden by various mechanisms, have found some validation in a pilot clinical trial in which AD patients treated for 6 months with rosiglitazone showed reduced attention and memory deficits [7]. In a second recent trial, the improvement in cognition after 6 months of rosiglitazone treatment was significant only in AD patients who did not have the ε4 allele of the apolipoprotein E [92], a genotype associate with a higher risk to develop AD. Similarly, the better neurological outcome reported after administration of PPAR-γ ligands in experimental stroke models are consistent with the result of a small clinical trial reporting that patients with diabetes receiving pioglitazone or rosiglitazone had an improved functional recovery after stroke compare to patients, who have not used any TZD [93]. Furthermore, a large clinical trial has demonstrated that pioglitazone reduced the combined risk of heart attack, stroke, and death in high risk type 2 diabetes patients [94].

The clinical use of PPAR-γ agonists in MS and ASL remains poorly investigated. Nonetheless, in a case report, pioglitazone treatment of an MS patient resulted in increased body weight and improved motor strength and coordination [27]. A first clinical trial for the use of pioglitazone in ALS started in Germany in late 2007.

Although PPAR-γ synthetic ligands such as TZDs and NSAIDs appear to be very promising drugs to treat severe human diseases, from cancer to metabolic diseases to brain diseases, several open issues still need to be examined. Among these, the toxic effect associated with some PPAR-γ agonists and their blood-brain-barrier permeability, which are at present still matter of controversies. A deep knowledge of the molecular mechanisms evoked by PPAR-γ ligands either dependent or independent of the receptor activation and of the dependence of such effects on the specific cell type is mandatory for the development of PPAR-γ drugs with increasing efficacy and safety.

ABBREVIATIONS

15d-PJ_2: 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2
AD: Alzheimer disease
ALS: Amyotrophic lateral sclerosis
AP-1: Activator protein-1
CNS: Central nervous system
COX: Cyclooxygenase
DBD: DNA-binding domain
EAE: Experimental autoimmune encephalomyelitis
HODE: Hydroxy octadecadienoic acids
IFN: Interferon
IL: Interleukin
iNOS: Inducible nitric oxide synthase
JAK: Janus activated kinases
LBD: Ligand-binding domain
LPS: Lipopolysaccharide
MAPK: Mitogen-activated protein kinase
MCP-1: Monocyte chemoattractrant protein-1
MHC: Major histocompatibility complex
MS: Multiple sclerosis
NFκB: Nuclear factor κB
NO: Nitric oxide
NSAIDs: Nonsteroidal anti-inflammatory drugs
oxLDL: Oxidized low-density lipoprotein
PD: Parkinson disease
PPAR: Peroxisome proliferator-activated receptor
PPRE: Peroxisome proliferator response Elements
RXR: Retinoid X-receptor
SOCS: Suppressor of cytokine signalling
STAT: Signal transducer and activators of transcription
Th: T helper cell
TNF: Tumour necrosis factor
TZDs: Thiazolidinediones

REFERENCES

[1] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[2] P. Escher and W. Wahli, "Peroxisome proliferator-activated receptors: insight into multiple cellular functions," *Mutation Research*, vol. 448, no. 2, pp. 121–138, 2000.

[3] M. Stumvoll, N. Stefan, A. Fritsche, et al., "Interaction effect between common polymorphisms in PPARγ2 (Pro12Ala) and insulin receptor substrate 1 (Gly972Arg) on insulin sensitivity," *Journal of Molecular Medicine*, vol. 80, no. 1, pp. 33–38, 2002.

[4] T. M. Willson, M. H. Lambert, and S. A. Kliewer, "Peroxisome proliferator-activated receptor γ and metabolic disease," *Annual Review of Biochemistry*, vol. 70, pp. 341–367, 2001.

[5] M. T. Heneka, G. E. Landreth, and M. Hüll, "Drug Insight: effects mediated by peroxisome proliferator-activated receptor-γ in CNS disorders," *Nature Clinical Practice Neurology*, vol. 3, no. 9, pp. 496–504, 2007.

[6] W. A. Pedersen, P. J. McMillan, J. J. Kulstad, J. B. Leverenz, S. Craft, and G. R. Haynatzki, "Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice," *Experimental Neurology*, vol. 199, no. 2, pp. 265–273, 2006.

[7] G. S. Watson, B. A. Cholerton, M. A. Reger, et al., "Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study," *American Journal of Geriatric Psychiatry*, vol. 13, no. 11, pp. 950–958, 2005.

[8] Q. Jiang, M. T. Heneka, and G. E. Landreth, "The role of peroxisome proliferator-activated receptor-γ (PPARγ) in Alzheimer's disease: therapeutic implications," *CNS Drugs*, vol. 22, no. 1, pp. 1–14, 2008.

[9] T. Breidert, J. Callebert, M. T. Heneka, G. Landreth, J. M. Launay, and E. C. Hirsch, "Protective action of the peroxisome proliferator-activated receptor-γ agonist pioglitazone in a mouse model of Parkinson's disease," *Journal of Neurochemistry*, vol. 82, no. 3, pp. 615–624, 2002.

[10] B. Schütz, J. Reimann, L. Dumitrescu-Ozimek, et al., "The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice," *Journal of Neuroscience*, vol. 25, no. 34, pp. 7805–7812, 2005.

[11] T. Shimazu, I. Inoue, N. Araki, et al., "A peroxisome proliferator-activated receptor-γ agonist reduces infarct size in transient but not in permanent ischemia," *Stroke*, vol. 36, no. 2, pp. 353–359, 2005.

[12] S. Sundararajan, J. L. Gamboa, N. A. Victor, E. W. Wanderi, W. D. Lust, and G. E. Landreth, "Peroxisome proliferator-activated receptor-γ ligands reduce inflammation and infarction size in transient focal ischemia," *Neuroscience*, vol. 130, no. 3, pp. 685–696, 2005.

[13] K. Tureyen, R. Kapadia, K. K. Bowen, et al., "Peroxisome proliferator-activated receptor-γ agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents," *Journal of Neurochemistry*, vol. 101, no. 1, pp. 41–56, 2007.

[14] X. Zhao, Y. Zhang, R. Strong, J. C. Grotta, and J. Aronowski, "15d-prostaglandin J_2 activates peroxisome proliferator-activated receptor-γ, promotes expression of catalase, and reduces inflammation, behavioural dysfunction, and neuronal loss after intracerebral haemorrhage in rats," *Journal of Cerebral Blood Flow & Metabolism*, vol. 26, no. 6, pp. 811–820, 2006.

[15] M. Blanco, M. A. Moro, A. Dávalos, et al., "Increased plasma levels of 15-deoxy Δ prostaglandin J_2 are associated with good outcome in acute atherothrombotic ischemic stroke," *Stroke*, vol. 36, no. 6, pp. 1189–1194, 2005.

[16] S.-W. Park, J.-H. Yi, G. Miranpuri, et al., "Thiazolidinedione class of peroxisome proliferator-activated receptor γ agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1002–1012, 2007.

[17] A. E. Lovett-Racke, R. Z. Hussain, S. Northrop, et al., "Peroxisome proliferator-activated receptor α agonists as therapy for autoimmune disease," *Journal of Immunology*, vol. 172, no. 9, pp. 5790–5798, 2004.

[18] P. E. Polak, S. Kalinin, C. Dello Russo, et al., "Protective effects of a peroxisome proliferator-activated receptor-β/δ agonist in experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 168, no. 1-2, pp. 65–75, 2005.

[19] M. Niino, K. Iwabuchi, S. Kikuchi, et al., "Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-γ," *Journal of Neuroimmunology*, vol. 116, no. 1, pp. 40–48, 2001.

[20] C. Natarajan and J. J. Bright, "Peroxisome proliferator-activated receptor-γ agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signalling and Th1 differentiation," *Genes & Immunity*, vol. 3, no. 2, pp. 59–70, 2002.

[21] A. Diab, C. Deng, J. D. Smith, et al., "Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis," *Journal of Immunology*, vol. 168, no. 5, pp. 2508–2515, 2002.

[22] D. L. Feinstein, E. Galea, V. Gavrilyuk, et al., "Peroxisome proliferator-activated receptor-γ agonists prevent experimental autoimmune encephalomyelitis," *Annals of Neurology*, vol. 51, no. 6, pp. 694–702, 2002.

[23] H. P. Raikwar, G. Muthian, J. Rajasingh, C. N. Johnson, and J. J. Bright, "PPARγ antagonists reverse the inhibition of neural antigen-specific Th1 response and experimental allergic encephalomyelitis by Ciglitazone and 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2," *Journal of Neuroimmunology*, vol. 178, no. 1-2, pp. 76–86, 2006.

[24] S. Dasgupta, A. Roy, M. Jana, D. M. Hartley, and K. Pahan, "Gemfibrozil ameliorates relapsing-remitting experimental autoimmune encephalomyelitis independent of peroxisome proliferator-activated receptor-α," *Molecular Pharmacology*, vol. 72, no. 4, pp. 934–946, 2007.

[25] M. Peiris, G. R. Monteith, S. J. Roberts-Thomson, and P. J. Cabot, "A model of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice for the characterisation of intervention therapies," *Journal of Neuroscience Methods*, vol. 163, no. 2, pp. 245–254, 2007.

[26] F. Malchiodi-Albedi, A. Matteucci, A. Bernardo, and L. Minghetti, "PPAR-γ, microglial cells and ocular inflammation: new venues for potential therapeutic approaches," *PPAR Research*, vol. 2008, Article ID 295784, 12 pages, 2008.

[27] H. A. Pershadsingh, M. T. Heneka, R. Saini, N. M. Amin, D. J. Broeske, and D. L. Feinstein, "Effect of pioglitazone treatment in a patient with secondary multiple sclerosis," *Journal of Neuroinflammation*, vol. 1,, pp. 1–4, 2004.

[28] M. Ricote and C. K. Glass, "PPARs and molecular mechanisms of transrepression," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 926–935, 2007.

[29] D. L. Feinstein, A. Spagnolo, C. Akar, et al., "Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key?" *Biochemical Pharmacology*, vol. 70, no. 2, pp. 177–188, 2005.

[30] R. Mukherjee, L. Jow, G. E. Croston, and J. R. Paterniti Jr., "Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists," *Journal of Biological Chemistry*, vol. 272, no. 12, pp. 8071–8076, 1997.

[31] L. Fajas, D. Auboeuf, E. Raspé, et al., "The organisation, promoter analysis, and expression of the human PPARγ gene," *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[32] L. Fajas, J.-C. Fruchart, and J. Auwerx, "PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter," *FEBS Letters*, vol. 438, no. 1-2, pp. 55–60, 1998.

[33] S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann, "A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation," *Cell*, vol. 83, no. 5, pp. 813–819, 1995.

[34] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[35] A. Bernando and L. Minghetti, "PPAR-γ agonists as regulators of microglial activation and brain inflammation," *Current Pharmaceutical Design*, vol. 12, no. 1, pp. 93–109, 2006.

[36] A. Bernardo, M. A. Ajmone-Cat, L. Gasparini, E. Ongini, and L. Minghetti, "Nuclear receptor peroxisome proliferator-activated receptor-γ is activated in rat microglial cells by the anti-inflammatory drug HCT1026, a derivative of flurbiprofen," *Journal of Neurochemistry*, vol. 92, no. 4, pp. 895–903, 2005.

[37] A. Bernardo, L. Gasparini, E. Ongini, and L. Minghetti, "Dynamic regulation of microglial functions by the non-steroidal anti-inflammatory drug NCX 2216: implications for chronic treatments of neurodegenerative diseases," *Neurobiology of Disease*, vol. 22, no. 1, pp. 25–32, 2006.

[38] J. Trotter, "The development of myelin-forming glia: studies with primary cell cultures and immortalized cell lines," *Perspectives on Developmental Neurobiology*, vol. 1, no. 3, pp. 149–154, 1993.

[39] S. A. Back, N. L. Luo, N. S. Borenstein, J. M. Levine, J. J. Volpe, and H. C. Kinney, "Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury," *Journal of Neuroscience*, vol. 21, no. 4, pp. 1302–1312, 2001.

[40] S. A. Back, B. H. Han, N. L. Luo, et al., "Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia," *Journal of Neuroscience*, vol. 22, no. 2, pp. 455–463, 2002.

[41] A. Bernardo, A. Greco, G. Levi, and L. Minghetti, "Differential lipid peroxidation, Mn superoxide, and bcl-2 expression contribute to the maturation-dependent vulnerability of oligodendrocytes to oxidative stress," *Journal of Neuropathology & Experimental Neurology*, vol. 62, no. 5, pp. 509–519, 2003.

[42] M. Zawadzka and R. J. M. Franklin, "Myelin regeneration in demyelinating disorders: new developments in biology and clinical pathology," *Current Opinion in Neurology*, vol. 20, no. 3, pp. 294–298, 2007.

[43] J. G. Granneman, R. Skoff, and X. Yang, "Member of the peroxisome proliferator-activated receptor family of transcription factors is differentially expressed by oligodendrocytes," *Journal of Neuroscience Research*, vol. 51, no. 5, pp. 563–573, 1998.

[44] I. Saluja, J. G. Granneman, and R. P. Skoff, "PPAR δ agonists stimulate oligodendrocyte differentiation in tissue culture," *Glia*, vol. 33, no. 3, pp. 191–204, 2001.

[45] A. D. Roth, A. V. Leisewitz, J. E. Jung, et al., "PPAR γ activators induce growth arrest and process extension in B12 oligodendrocyte-like cells and terminal differentiation of cultured oligodendrocytes," *Journal of Neuroscience Research*, vol. 72, no. 4, pp. 425–435, 2003.

[46] C. M. Kassmann, C. Lappe-Siefke, M. Baes, et al., "Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes," *Nature Genetics*, vol. 39, no. 8, pp. 969–976, 2007.

[47] G. Calamandrei, A. P. Venerosi, A. Valanzano, et al., "Increased brain levels of F2-isoprostane are an early marker of behavioral sequels in a rat model of global perinatal asphyxia," *Pediatric Research*, vol. 55, no. 1, pp. 85–92, 2004.

[48] P. Piscopo, A. Bernardo, G. Calamandrei, et al., "Altered expression of cyclooxygenase-2, presenilins and oxygen radical scavenging enzymes in a rat model of global perinatal asphyxia," *Experimental Neurology*, vol. 209, no. 1, pp. 192–198, 2008.

[49] Z. Xiang, T. Lin, and S. A. Reeves, "15d-PGJ_2 induces apoptosis of mouse oligodendrocyte precursor cells," *Journal of Neuroinflammation*, vol. 4, article 18, pp. 1–10, 2007.

[50] A. Bernardo, M. A. Ajmone-Cat, G. Levi, and L. Minghetti, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 regulates the functional state and the survival of microglial cells through multiple molecular mechanisms," *Journal of Neurochemistry*, vol. 87, no. 3, pp. 742–751, 2003.

[51] M. Nedergaard and U. Dirnagl, "Role of glial cells in cerebral ischemia," *Glia*, vol. 50, no. 4, pp. 281–286, 2005.

[52] C. Farina, F. Aloisi, and E. Meinl, "Astrocytes are active players in cerebral innate immunity," *Trends in Immunology*, vol. 28, no. 3, pp. 138–145, 2007.

[53] T. E. Cullingford, K. Bhakoo, S. Peuchen, C. T. Dolphin, R. Patel, and J. B. Clark, "Distribution of mRNAs encoding the peroxisome proliferator-activated receptor α, β, and γ and the retinoid X receptor α, β, and γ in rat central nervous system," *Journal of Neurochemistry*, vol. 70, no. 4, pp. 1366–1375, 1998.

[54] L. Cristiano, A. Bernardo, and M. P. Cerù, "Peroxisome proliferator-activated receptors (PPARs) and peroxisomes in rat cortical and cerebellar astrocytes," *Journal of Neurocytology*, vol. 30, no. 8, pp. 671–683, 2001.

[55] C. Dello Russo, V. Gavrilyuk, G. Weinberg, et al., "Peroxisome proliferator-activated receptor γ thiazolidinedione agonists increase glucose metabolism in astrocytes," *Journal of Biological Chemistry*, vol. 278, no. 8, pp. 5828–5836, 2003.

[56] C. Romera, O. Hurtado, J. Mallolas, et al., "Ischemic preconditioning reveals that GLT1/EAAT2 glutamate transporter is a novel PPARγ target gene involved in neuroprotection," *Journal of Cerebral Blood Flow & Metabolism*, vol. 27, no. 7, pp. 1327–1338, 2007.

[57] M. Toyomoto, M. Ohta, K. Okumura, et al., "Prostaglandins are powerful inducers of NGF and BDNF production in mouse astrocyte cultures," *FEBS Letters*, vol. 562, no. 1–3, pp. 211–215, 2004.

[58] R. Luna-Medina, M. Cortes-Canteli, M. Alonso, A. Santos, A. Martínez, and A. Perez-Castillo, "Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor γ activation," *Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21453–21462, 2005.

[59] R. Luna-Medina, M. Cortes-Canteli, S. Sanchez-Galiano, et al., "NP031112, a thiadiazolidinone compound, prevents inflammation and neurodegeneration under excitotoxic conditions: potential therapeutic role in brain disorders," *Journal of Neuroscience*, vol. 27, no. 21, pp. 5766–5776, 2007.

[60] N. Janabi, "Selective inhibition of cyclooxygenase-2 expression by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in activated human astrocytes, but not in human brain macrophages," *Journal of Immunology*, vol. 168, no. 9, pp. 4747–4755, 2002.

[61] A. M. Lennon, M. Ramaugé, A. Dessouroux, and M. Pierre, "MAP kinase cascades are activated in astrocytes and preadipocytes by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and the thiazolidinedione ciglitazone through peroxisome proliferator activator receptor γ-independent mechanisms involving reactive oxygenated species," *Journal of Biological Chemistry*, vol. 277, no. 33, pp. 29681–29685, 2002.

[62] E. J. Park, S. Y. Park, E.-H. Joe, and I. Jou, "15d-PGJ_2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia," *Journal of Biological Chemistry*, vol. 278, no. 17, pp. 14747–14752, 2003.

[63] J. Xu and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia," *Journal of Immunology*, vol. 178, no. 3, pp. 1904–1913, 2007.

[64] P. D. Storer, J. Xu, J. Chavis, and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroimmunology*, vol. 161, no. 1-2, pp. 113–122, 2005.

[65] A. S. Paintlia, M. K. Paintlia, I. Singh, and A. K. Singh, "IL-4-induced peroxisome proliferator-activated receptor γ activation inhibits NF-κB *trans* activation in central nervous system (CNS) glial cells and protects oligodendrocyte progenitors under neuroinflammatory disease conditions: implication for CNS-demyelinating diseases," *Journal of Immunology*, vol. 176, no. 7, pp. 4385–4398, 2006.

[66] Y. Kitamura, T. Taniguchi, H. Kimura, Y. Nomura, and P. J. Gebicke-Haerter, "Interleukin-4-inhibited mRNA expression in mixed rat glial and in isolated microglial cultures," *Journal of Neuroimmunology*, vol. 106, no. 1-2, pp. 95–104, 2000.

[67] D. J. Loane, B. F. Deighan, R. M. Clarke, R. J. Griffin, A. M. Lynch, and M. A. Lynch, "Interleukin-4 mediates the neuroprotective effects of rosiglitazone in the aged brain," *Neurobiology of Aging*. In press.

[68] N. K. Phulwani, D. L. Feinstein, V. Gavrilyuk, C. Akar, and T. Kielian, "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," *Journal of Neurochemistry*, vol. 99, no. 5, pp. 1389–1402, 2006.

[69] M.-G. Zurich, S. Lengacher, O. Braissant, F. Monnet-Tschudi, L. Pellerin, and P. Honegger, "Unusual astrocyte reactivity caused by the food mycotoxin ochratoxin A in aggregating rat brain cell cultures," *Neuroscience*, vol. 134, no. 3, pp. 771–782, 2005.

[70] G. W. Kreutzberg, "Microglia: a sensor for pathological events in the CNS," *Trends in Neurosciences*, vol. 19, no. 8, pp. 312–318, 1996.

[71] A. Nimmerjahn, F. Kirchhoff, and F. Helmchen, "Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo," *Science*, vol. 308, no. 5726, pp. 1314–1318, 2005.

[72] V. H. Perry, C. Cunningham, and C. Holmes, "Systemic infections and inflammation affect chronic neurodegeneration," *Nature Reviews Immunology*, vol. 7, no. 2, pp. 161–167, 2007.

[73] T. Kielian and P. D. Drew, "Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation," *Journal of Neuroscience Research*, vol. 71, no. 3, pp. 315–325, 2002.

[74] T. V. Petrova, K. T. Akama, and L. J. Van Eldik, "Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 8, pp. 4668–4673, 1999.

[75] T. Koppal, T. V. Petrova, and L. J. Van Eldik, "Cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 acts as a general inhibitor of inflammatory responses in activated BV-2 microglial cells," *Brain Research*, vol. 867, no. 1-2, pp. 115–121, 2000.

[76] A. Bernardo, G. Levi, and L. Minghetti, "Role of the peroxisome proliferator-activated receptor-γ (PPAR-γ) and its natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in the regulation

of microglial functions," *European Journal of Neuroscience*, vol. 12, no. 7, pp. 2215–2223, 2000.

[77] P. D. Drew and J. A. Chavis, "The cyclopentone prostaglandin 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 represses nitric oxide, TNF-α, and IL-12 production by microglial cells," *Journal of Neuroimmunology*, vol. 115, no. 1-2, pp. 28–35, 2001.

[78] T. Kielian, M. McMahon, E. D. Bearden, A. C. Baldwin, P. D. Drew, and N. Esen, "S. *aureus*-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2)," *Journal of Neurochemistry*, vol. 90, no. 5, pp. 1163–1172, 2004.

[79] E. J. Kim, K. J. Kwon, J.-Y. Park, S. H. Lee, C.-H. Moon, and E. J. Baik, "Effects of peroxisome proliferator-activated receptor agonists on LPS-induced neuronal death in mixed cortical neurons: associated with iNOS and COX-2," *Brain Research*, vol. 941, no. 1-2, pp. 1–10, 2002.

[80] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of β- amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists," *Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[81] L. Gasparini, E. Ongini, D. Wilcock, and D. Morgan, "Activity of flurbiprofen and chemically related anti-inflammatory drugs in models of Alzheimer's disease," *Brain Research Reviews*, vol. 48, no. 2, pp. 400–408, 2005.

[82] P. T. Jantzen, K. E. Connor, G. DiCarlo, et al., "Microglial activation and β-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice," *Journal of Neuroscience*, vol. 22, no. 6, pp. 2246–2254, 2002.

[83] D. S. Straus, G. Pascual, M. Li, et al., "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 9, pp. 4844–4849, 2000.

[84] T. A. Kennedy, C. J. Smith, and L. J. Marnett, "Investigation of the role of cysteines in catalysis by prostaglandin endoperoxide synthase," *Journal of Biological Chemistry*, vol. 269, no. 44, pp. 27357–27364, 1994.

[85] K. M. Maxey, E. Hessler, J. MacDonald, and L. Hitchingham, "The nature and composition of 15-deoxy-$\Delta^{12,14}PGJ_2$," *Prostaglandins & Other Lipid Mediators*, vol. 62, no. 1, pp. 15–21, 2000.

[86] T. Shibata, T. Yamada, M. Kondo, et al., "An endogenous electrophile that modulates the regulatory mechanism of protein turnover: inhibitory effects of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on proteasome," *Biochemistry*, vol. 42, no. 47, pp. 13960–13968, 2003.

[87] S. Hortelano, A. Castrillo, A. M. Alvarez, and L. Bosca, "Contribution of cyclopentenone prostaglandins to the resolution of inflammation through the potentiation of apoptosis in activated macrophages," *Journal of Immunology*, vol. 165, no. 11, pp. 6525–6531, 2000.

[88] S. G. Harris, J. Padilla, L. Koumas, D. Ray, and R. P. Phipps, "Prostaglandins as modulators of immunity," *Trends in Immunology*, vol. 23, no. 3, pp. 144–150, 2002.

[89] M. Kondo, T. Shibata, T. Kumagai, et al., "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2: the endogenous electrophile that induces neuronal apoptosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 11, pp. 7367–7372, 2002.

[90] T. Zander, J. A. Kraus, C. Grommes, et al., "Induction of apoptosis in human and rat glioma by agonists of the nuclear receptor PPARγ," *Journal of Neurochemistry*, vol. 81, no. 5, pp. 1052–1060, 2002.

[91] S. G. Harris and R. P. Phipps, "The nuclear receptor PPAR γ is expressed by mouse T lymphocytes and PPAR γ agonists induce apoptosis," *European Journal of Immunology*, vol. 31, no. 4, pp. 1098–1105, 2001.

[92] M. E. Risner, A. M. Saunders, J. F. B. Altman, et al., "Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease," *The Pharmacogenomics Journal*, vol. 6, no. 4, pp. 246–254, 2006.

[93] J. Lee and M. Reding, "Effects of thiazolidinediones on stroke recovery: a case-matched controlled study," *Neurochemical Research*, vol. 32, no. 4-5, pp. 635–638, 2007.

[94] J. A. Dormandy, B. Charbonnel, D. J. A. Eckland, et al., "Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial," *The Lancet*, vol. 366, no. 9493, pp. 1279–1289, 2005.

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Review Article

PPAR Regulation of Inflammatory Signaling in CNS Diseases

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Central nervous system (CNS) is an immune privileged site, nevertheless inflammation associates with many CNS diseases. Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear hormone receptors that regulate immune and inflammatory responses. Specific ligands for PPARα, γ, and δ isoforms have proven effective in the animal models of multiple sclerosis (MS), Alzheimer's disease, Parkinson's disease, and trauma/stroke, suggesting their use in the treatment of neuroinflammatory diseases. The activation of NF-κB and Jak-Stat signaling pathways and secretion of inflammatory cytokines are critical in the pathogenesis of CNS diseases. Interestingly, PPAR agonists mitigate CNS disease by modulating inflammatory signaling network in immune cells. In this manuscript, we review the current knowledge on how PPARs regulate neuroinflammatory signaling networks in CNS diseases.

1. INTRODUCTION

The central nervous system (CNS) was thought to be an immune privileged site due to the ability of blood-brain-barrier (BBB) to shield immune cell entry and protect from the constantly changing circulatory milieu. Nevertheless, activated immune cells readily traverse the BBB, secrete inflammatory cytokines, and mediate many CNS diseases. Neuroinflammatory diseases present major challenges to the health care system and impose substantial economic costs around the world. Current treatments targeting clinical symptoms of CNS diseases have modest therapeutic values in patients. Significant progress has been made in recent years in developing therapeutic strategies for the treatment of neuroinflammatory diseases.

2. NEUROINFLAMMATORY DISEASES

The innate and adaptive immunity evoked during infection in the CNS often leads to the development of neuroinflammatory diseases [1–3]. The mounting evidence suggests that neuroinflammatory diseases such as multiple sclerosis (MS), Alzheimer's disease (AD), trauma, and ischemia/stroke can occur in the absence of infection. MS is an inflammatory demyelinating disease of the CNS with clinical symptoms ranging from pain to paralysis and the patients becoming wheel-chair bound for rest of their lives [4]. Although the etiology of MS is not known, it is generally viewed as a neural antigen-specific T cell-mediated autoimmune disease [4–6]. Experimental allergic encephalomyelitis (EAE) is an autoimmune disease model of MS, commonly used to study the mechanism of disease pathogenesis and to test the efficacy of potential therapeutic agents for the treatment of MS. In AD, the deposition of beta-amyloid (Aβ) and plaque formation in the CNS associate with inflammation resulting in neuronal death, progressive deterioration of cognitive functions, and memory loss [7, 8]. Traumatic brain injury (TBI), spinal cord injury, and ischemic stroke also display neuroinflammation associated secondary tissue damage in the CNS [9, 10]. The pathogenesis of neuroinflammatory diseases involves the orchestrated interaction of immune cells resulting in tissue injury to the CNS [6, 11]. Although the exact mechanisms are not known, recent evidence suggests the use of peroxisome proliferator-activated receptor (PPAR) agonists in the treatment of neuroinflammatory diseases.

3. PPAR ISOFORMS AND THEIR LIGANDS

PPAR is a family of ligand-dependent nuclear hormone receptor transcription factors that play key roles in the

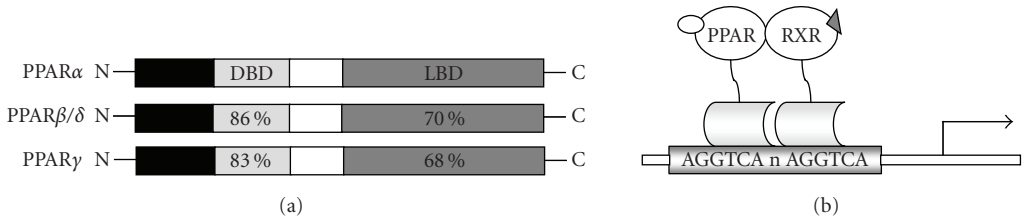

Figure 1: (a) Functional domains of PPAR isoforms. N, N-terminus; DBD: DNA-binding domain; LBD: ligand-binding domain. The numbers represent percentage identity to human PPARα. (b) PPAR/RXR binds to PPREDR-1 promoter regions. Binding of agonists leads to heterodimerization, recruitment of coactivator and transcriptional activation of target genes.

regulation of immune and inflammatory responses [12]. Structure-function analyses revealed that PPARs are composed of a DNA-binding domain (DBD) linked to the C-terminal ligand-binding domain (LBD) by a hinge region (Figure 1) [13, 14]. PPARs stimulate gene expression through binding to peroxisome-proliferator response elements (PPREs), present in the promoter regions of the target genes. In the absence of ligands, the heterodimers physically associate with corepressors and suppress gene transcription [14, 15]. Upon ligand binding, the coactivators replace corepressors and activate gene expression [16, 17]. PPARα, PPARβ/δ, and PPARγ are three structurally homologous isotypes found in various species which display distinct physiological and pharmacological functions [18]. The PPARα is expressed in liver, kidney, intestine, heart, skeletal muscle, adrenal gland, pancreas, and brain. PPARα is involved in acetylcholine metabolism, excitatory neurotransmission, and oxidative stress defense [19]. PPARα also regulates lipid metabolism and energy homeostasis through its ability to stimulate the breakdown of fatty acids and cholesterol, driving gluconeogenesis and reduction in serum triglyceride levels [19]. While polyunsaturated fatty acids activate all three isoforms of PPARs with different affinities, each isotype has its own ligand binding property [20]. Fibrates, WY14643, and GW7647 are PPARα agonists commonly used for the treatment of hypertriglyceridemia [19].

PPARβ/δ is ubiquitously expressed in all cell types including immature oligodendrocytes and promotes differentiation and myelination in the CNS [21–23]. PPARβ/δ null mice show an altered myelination of corpus callosum, suggesting its role in brain function [24]. PPARβ/δ regulates transcriptional activation of Acyl-CoA synthetase 2, a key enzyme in fatty acid utilization, suggesting its role in lipid metabolism in the brain. Prostagladin I_2, GW0742, GW501516, and GW7842 are PPARβ/δ agonists which induce fatty acid oxidation in muscle [25]. PPARγ expression is detected in adipose tissue intestinal mucosa, retina, skeletal muscle, heart, liver, and lymphoid organs [26]. PPARγ is expressed in microglia and astrocytes and regulates inflammation in the CNS [27, 28]. Eicosanoids and prostaglandin J2 (15d-PGJ2) are the naturally occurring PPARγ ligands, and thiazolidinedones (TZDs) including pioglitazone (Actos) and rosiglitazone (Avandia) are Food and Drug Administration (FDA) approved synthetic drugs for the treatment of type II diabetes [29]. Recent studies have shown the use of PPAR agonists in the treatment of many neuroinflammatory diseases.

4. THERAPEUTIC EFFECTS OF PPAR AGONISTS IN CNS DISEASES

The therapeutic effects of PPAR agonists have been tested in many different neuroinflammatory diseases (Table 1). The use of PPARγ agonists in the treatment of MS has been tested in EAE model by different groups [30–33]. In vivo treatment with synthetic PPARγ ligand, troglitazone, ameliorates EAE by reducing the infiltration of leukocytes in the CNS [34]. Two other studies also showed that in vivo treatment with PPARγ ligands, 15d-PGJ_2 and ciglitazone, ameliorates EAE [30, 31]. Oral treatment with pioglitazone inhibits chronic progressive and relapsing forms of EAE even when administered at the peak of disease [35, 36], suggesting their use of PPARγ agonists in the treatment of MS. PPARγ-deficient heterozygous mice develop an exacerbated EAE with increased CNS inflammation and demyelination [37]. A recent report also showed that PPARγ antagonists, bisphenol A diglycidyl ether (BADGE), and 2-chloro-5 nitro-N-(4 pyridyl) benzamide (T007) reversed the inhibition of EAE by PPARγ agonists, further suggesting the physiological role of PPARγ in the pathogenesis of EAE [38].

Epidemiological studies suggest a reduced risk of AD among the users of nonsteroidal anti-inflammatory drugs (NSAID) [39, 40]. Treatment with pioglitazone and rosiglitazone significantly reduced the lesion size, motor neuron loss, myelin loss, astrogliosis, microglial activation, and chronic thermal hyperalgesia in spinal cord injury [41]. In a rat model of AD induced by cortical Aβ injection, ciglitazone and pioglitazone suppressed the clinical symptoms significantly. In the amyloid precursor protein (APP) transgenic model of AD, treatment with pioglitazone reduced the plaque burden by affecting the production, clearance, and homeostasis of Aβ in the CNS [42]. A clinical trial involving 500 AD patients showed significant improvement in cognition following treatment with rosiglitazone for 6 months, suggesting its use in the treatment of AD [43]. Recent evidence also suggests that NSAIDs such as ibuprofen may delay or prevent the development of Parkinson's disease (PD) [44, 45]. Moreover, PPARγ is expressed in the CNS of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model of PD [24] and treatment with pioglitazone protected the animals from neuronal cell death [46]. Similar results

Table 1: Role of PPARs in the regulation of neuroinflammatory diseases.

CNS disease	Inflammatory response	Effect of PPAR agonists
Multiple sclerosis	Activation of macrophage, microglia and dendritic cells; infiltration of Th1/Th17 cells in the CNS; induction of NF-κB and Jak-Stat pathway and release of IL-12, IFNγ, IL-17 and other cytokines in the CNS	PPARα, δ and γ agonists ameliorate EAE by inhibiting inflammation
Alzheimer's disease	Beta-amyloid (Aβ) accumulation leads to CNS inflammation via TNFα and NF-κB pathway and secretion of inflammatory cytokines	PPARγ ligands reduce neuronal loss in animal models of AD
Infection	During bacterial, viral, fungal and parasitic infection, activated APC and T cells release TNFα, IFNγ, iNOS, IL-2, IL-6 and induce inflammation via NF-κB, Stat and AP-1 signaling pathways	PPAR agonists regulate infection associated inflammation
Trauma	CNS injury results in the activation of resident microglia and astrocytes resulting inflammation through secretion of TNFα, prostaglandin and COX-2 and mediate inflammation via NF-κB, Stat1 and AP-1 pathways	PPARα, δ and γ ligands regulate inflammatory response in trauma
Ischemia/stroke	Ischemic stroke associates with recruitment and activation of macrophages and neutrophils via increased expression of VCAM-1, ICAM-1, IL-6, IL-8 and COX-2 through Stat-1	PPARγ ligands reduce the infarct size in animal models

were also generated using lipopolysaccharide (LPS)-induced inflammation model of dopaminergic neurodegeneration in rat, where pioglitazone treatment effectively reduced inflammation, oxidative stress, and restored mitochondrial function [47]. Treatment with pioglitazone also extends the survival of superoxide dismutase-1 (SOD1-G93A) transgenic animal model of amylotrophic lateral sclerosis (ALS) [36, 48–51].

The effects of PPAR agonists in reducing deleterious inflammatory responses suggest their use in the treatment of trauma, spinal cord injury, and stroke. Experimental evidence suggests that the Pro12Ala polymorphism of PPARγ2 is associated with a reduced risk for ischemic stroke [52] and treatment with TZDs and 15d-PGJ2 cause neuroprotection in animal models of stroke. Treatment with PPARγ agonists also reduce the infarct volumes and improve sensorimotor function in a rodent model of middle cerebral artery occlusion (MCAO) [53, 54]. Similar effects were observed following oral or intracerebrovascular administration of PPARγ agonists [55, 56]. TZD-unrelated PPARγ agonist L-796,449 decreases infarct size and improves neurological scores after MCAO in the rat brain [57]. Treatment with PPARγ antagonist T0070907 increased the infarct size and reversed rosiglitazone-induced protection after stroke. A small clinical trial has revealed improved functional recovery after stroke in diabetic patients receiving pioglitazone or rosiglitazone compared to patients not receiving TZD therapy [58]. A recent clinical trial demonstrated that pioglitazone significantly reduced the combined risk of myocardial infarction and stroke-associated death in high-risk patients with type 2 diabetes [59].

Recent studies have demonstrated the beneficial effects of PPARα agonists in the treatment of neuroinflammatory diseases. Oral treatment with gemfibrozil protects mice from EAE [60]. The tyrosine hydroxylase (TH)-positive SNpc cells express PPARα and in vivo treatment with PPARα agonist, fenofibrate, protects mice from MPTP-induced inflammation and neuronal loss. In vivo treatment with PPARα agonist, fenofibrate and WY-14643, reduced the infarct size in mouse models of stroke [61, 62]. This effect was absent in PPARα deficient mice, reinforcing receptor dependency of the observed effects . Treatment with PPARα agonist, fenofibrate, decreases the neurological deficit induced by traumatic brain injury (TBI) caused by lateral fluid percussion of brain in rats [63]. Fenofibrate also reduces brain edema and ICAM-1 expression and induces neurological recovery associated with a reduction of the brain lesion. Anti-inflammatory therapies showed neuroprotective effects after spinal cord injury in rodents [64, 65]. Moreover, oral treatment with selective PPARβ/δ agonist GW0742 exerted beneficial effects in the MOGp35-55-induced EAE model [66]. GW0742 reduced the severity of EAE even when administered at the peak of clinical disease [66]. PPAR β/δ null mice exhibit significantly greater infarct sizes than wild type animals suggesting its role in stroke [67].

5. PPAR AGONISTS REGULATE INFLAMMATORY CYTOKINES IN CNS DISEASES

The anti-inflammatory effects of PPARγ agonists have been extensively studied in CNS diseases (Table 2). While the inflammatory cytokines, IL-1β, IL-6, TNFα, IL-12, IL-23, IL-27, IFNγ, and IL-17, mediate the pathogenesis of CNS diseases, anti-inflammatory cytokines, IL-4, IFNβ, TGFβ, and IL-10, confer recovery in MS and its animal model, EAE [68–70]. In EAE model of MS, PPARγ agonists decrease

Table 2: Role of PPARs in the regulation of inflammatory signaling pathways in CNS diseases.

Tissue Distribution	PPAR Agonists	Effect and Mode of Action in CNS diseases
PPARα Expressed in liver, heart, kidney, large intestine, skeletal muscle and astrocytes. PPARα knockout mice develop severe LPS-induced inflammation	Palmitic acid, linoleic acid, stearic acid, palmitoleic acid, oleic acid, 8-HETE, Wy-14643, clofibrate, nafenopin, bezafibrate, fenofibrate	PPARα agonists inhibit Aβ induced expression of TNFα, IL-6, IL-4 and infiltration of $CD4^+$ T cells in the CNS of AD; reduce ICAM-1 expression and oxidative damage in stroke; protect MPTP-induced loss of neurons in PD; protect mice from EAE by inhibiting IFNγ, TNFα and IL-6 production in stroke, cerebral ischemia and MS models
PPARβ/δ Expressed ubiquitously in brain, adipose tissue and skin. PPARβ/δ knockout mice show reduced fat deposition	Prostacyclin, PGI2, GW0742, GW501516, GW7842, L165041	PPARβ/δ agonists reduce the severity of EAE and stroke by inhibiting NF-κB and Jak-Stat signaling pathways in immune cells from MS and stroke models
PPARγ Expressed in heart, muscle, colon, kidney, pancreas, spleen, macrophage, intestine, adipose tissue and liver. PPARγ knockouts are embryonically lethal	Prostaglandin J2, thiazolidinediones, pioglitazone, rosiglitazone, GW78456, WY14,643, GW7647	PPARγ agonists inhibit T-cell proliferation, IFNγ, IL-10 and IL-4 production through blocking NF-κB, AP-1 and Jak-Stat pathways in CNS diseases models of AD, PD, Trauma, MS, ALS, stroke and ischemia

the TNFα mRNA expression in antigen-specific T cell in vitro [71]. Other studies have shown that 15d-PGJ_2 inhibits EAE in association with inhibition of T-cell proliferation and secretion of inflammatory cytokines including IFNγ, IL-10, and IL-4 in culture [31–34]. PPARγ agonists, 15d-PGJ2 and ciglitazone, block IL-12 signaling through Jak-Stat pathway leading to Th1 differentiation in T cells. Pioglitazone also suppresses IFNγ secretion in spleen T cells following stimulation with MOGp35-55 in vitro [30]. The activation of resident glial cells and infiltration of leukocytes contribute to demyelination in EAE and MS. The chemokines and chemokine receptors promote the trafficking and entry of immune cells across blood-brain barrier into the CNS in EAE and MS [71–74]. Whereas, PPARγ agonists, troglitazone and pioglitazone, reduce the expression of MCP1 [33], IP-10 (CXCL3), MIG, I-TAC, MIP1α, and RANTES [27] that contribute to reduced infiltration of immune cells in the MOG-induced EAE [73, 74]. Moreover, the surface molecules such as MHC class II, CD40, CD28, and ICAM enhance the disease pathogenesis and CTLA4 inhibits EAE/MS [75]. Negative regulation of adhesion molecules may also account for reduced brain infiltration observed in PPARγ agonists treated EAE mice.

The immunomodulatory effects of PPARγ agonists were tested in human peripheral blood mononuclear cells (PBMCs). In vitro treatment of cells with pioglitazone, ciglitazone, and GW347845 abolished proliferation and cytokine secretion accompanied by DNA condensation and down-regulation of bcl-2, suggesting the induction of apoptosis in activated T lymphocytes. MS patients showed a decrease in the expression of PPARγ in immune cells and a reduction in the anti-inflammatory effects of pioglitazone when compared to healthy controls [35, 36, 76]. However, treatment of immune cells derived from diabetic patients with pioglitazone in vitro or by oral treatment in vivo increased the expression and DNA-binding activity of PPARγ [77]. In MS patients, pioglitazone increased the DNA binding activity of PPARγ and decreased the NF-κB activity by increasing IκBα. Activated microglial cells were significantly reduced at sites of neurodegeneration in pioglitazone-treated SOD1-G93A mice, as were the protein levels of COX-2 and iNOS. The mRNA levels of the suppressor of cytokine signaling 1 and 3 genes were also increased by pioglitazone [48], but their functional significance is not well known.

In vivo treatment with PPARγ agonists suppresses Aβ-evoked microglial activation and inflammatory cytokine expression, iNOS expression and NO production, and inhibition of COX-2 in Aβ-evoked animal models of AD and APP [42]. PPARγ agonists also suppress Aβ-mediated activation of microglia in vitro [40–43]. The expression of iNOS in neurons resulted in neuronal cell death which was prevented by activation of PPARγ in vitro and in vivo [42, 43]. Neuroinflammatory changes accompanied by activation of microglia and astrocytes and expression of TNFα, IL -1β, and iNOS play a pivotal role in PD [46]. An increase in infiltrating CD8+ T lymphocytes and IFNγ+ cells were also reported in the CNS with PD. Pioglitazone decreased microglia and astrocyte activation and reduced the number of iNOS-positive cells in the CNS [47]. In trauma, macrophages, and neutrophils are involved in the early stages of inflammation followed by leukocyte recruitment via VCAM-1, ICAM-1, IL-6, IL-8, and COX-2 [75]. The leukocytes and microglia mount inflammatory responses with elevated expression of cytokines, chemokines, adhesion molecules, iNOS, COX-2, and other inflammatory mediators that exacerbate the tissue damage [61]. Treatment with pioglitazone significantly reduced the induction of inflammatory genes, IL-1β, IL-6, monocyte chemoatractant protein-1, and intracellular adhesionmolecule-1. The PPARγ antagonist, 2-chloro-5-nitro-*N*-phenyl-benzamide (GW9662), prevented

the neuroprotective effect of pioglitazone [48], suggesting the involvement of PPARγ-dependent mechanisms in the regulation of inflammation and new therapeutic avenue for the treatment of MS.

The expression and activation of PPARα in T lymphocytes decreases IL-2 production and proliferation. PPARα-null mice show an augmented LPS-induced inflammatory response and oral treatment with gemfibrozil reduced CD4+ lymphocyte and macrophage infiltration into the CNS of mice with EAE. Several agonists of PPARα, including gemfibrozil and ciprofibrate, decreased murine lymphocyte proliferation in a concentration-dependent manner, in vitro [60]. The gemfibrozil and ciprofibrate-induced IL-4 production in murine and human lymphocytes, whereas IFNγ production was decreased. WY14,643, a synthetic PPARα agonist, reduced the IgG response in mice with EAE and impaired generation of IFNγ, TNFα, and IL-6 in response to MOG peptide in vitro. PPARα and PPARβ/δ are expressed in astrocytes, while the latter are present more in oligodendrocytes, thus playing a role in the process of remyelination [66]. In AD, the neuroinflammatory components include resident microglia, astrocyte, the complement system, cytokines, and chemokines. Microglia and astrocytes generate beta-amyloid protein that stimulate proinflammatory cytokines in AD brain. PPARα agonists inhibitAβ-stimulated expression of TNFα and IL-6 in a dose dependent manner [40, 42]. In trauma and spinal cord injury-induced edema, neutrophil infiltration and immunoreactivity to TNFα were augmented with a worsened recovery of limb function in PPARα knockout than wild type mice. CNS injury leads to rapid recruitment of microglia, macrophage, and astrocytes that secrete IL-1, TNFα, iNOS, PGs, and COX-2 [63]. Fenofibrate promotes neurological recovery by decreasing iNOS, COX2, MMP9 expression, and antioxidant effect in TBI. Although PPAR agonists inhibit neuroinflammation in many CNS diseases, their modes of action are not well characterized.

6. PPAR AGONISTS REGULATE IL-12 FAMILY CYTOKINES IN CNS DISEASES

IL-12, IL-23, and IL-27 are three IL-12 family cytokines produced by macrophage, microglia, and dendritic cells in the CNS. IL-12 is a 70 kD heterodimeric cytokine composed of p40 and p35 subunits encoded by two different genes that play a critical role in the differentiation of neural antigen-specific Th1 cells in EAE [78, 79]. We and others have shown earlier that in vivo treatment with neutralizing anti-IL-12p40 antibody prevents EAE [79]. Furthermore, therapeutic intervention of IL-12-signaling was effective in preventing EAE. We have shown that PPARγ agonists inhibit IL-12 production, IL-12 signaling, and differentiation of Th1 cells in EAE [30]. We have also shown that PPARγ-deficient heterozygous mice develop an exacerbated EAE in association with an augmented Th1 response [37], suggesting a physiological role for PPARγ in the regulation of IL-12/IFNγ axis in CNS demyelination. IL-23 is a heterodimeric cytokine composed of a common IL-12p40 subunit and an IL-23p19 subunit specific to IL-23 encoded by two different genes [70]. Signaling through its receptor, composed of IL-12Rβ1 and IL-23R, IL-23 induces the activation of Jak-Stat pathways and differentiation of IL-17 producing (Th17) cells from memory Th1 cells, leading to the pathogenesis of EAE [80]. Targeted disruption of IL-23p19 in mice was effective in preventing the pathogenesis of EAE [70, 81] and suggested that the IL-23/IL-17 axis plays a critical role in the pathogenesis of CNS inflammation and demyelination. Although IL-6 and TGFβ [82] are important mediators of Th17 differentiation in culture, their physiological role in activating Th17 cells in CNS disease is not known (Figure 2).

IL-27 is another heterodimeric cytokine consisting of EBI3 and p28 encoded by two different genes. IL-27 receptor is composed of WSX-1 and gp130 molecules that mediate IL-27-induced activation of the Jak-Stat pathway in naive CD4+ T cells [83]. In vivo treatment with anti-IL-27 antibody ameliorates EAE, suggesting its role in the pathogenesis of Th1 cell-mediated autoimmune diseases. Recent studies have also shown that IL-27 and IFNγ are potent inducers of T-bet, a T-box protein transcription factor, in T cells. Targeted disruption of T-bet or siRNA inhibition of T-bet was sufficient to prevent the pathogenesis of EAE, suggesting the critical role of IL-27/IFNγ/T-bet axis in the pathogenesis of demyelination [84]. PPARγ agonists regulate IL-27/IFNγ/T-bet axis in EAE. Interestingly, recent studies have shown that EBI3 can also heterodimerize with IL-12p35 to form IL-35 in CD4+-Foxp3+ regulatory T cells and functions as a potent anti-inflammatory cytokine [85]. Although PPARγ has been shown to upregulate Treg cells in vitro [86], the role of PPAR in the development of Treg cells or production of IL-35 in EAE/MS or other CNS diseases is not known.

7. PPAR AGONISTS REGULATE NF-κB SIGNALING PATHWAYS IN CNS DISEASES

The IL-12 family cytokines are produced by macrophage, microglia, and dendritic cells in response to autoantigens, TLR ligands, and CD40 ligands [87]. In earlier studies, we and others have shown that autoimmune cells secrete IL-12 in response to antigens and that this response was inhibited by treatment with PPARγ agonists [30]. PPARγ agonists also inhibit LPS and CD40L-induced secretion of IL-12 from macrophage, microglia, and dendritic cells. The induction of IL-12/IL-23 gene expression involves activation of the NF-κB signaling pathway in antigen-presenting cells [88]. NF-κB is a heterodimeric transcription factor composed of p50 and p65 subunits from the Rel family of proteins. It is sequestered in the cytoplasm as an inactive complex when associated with its inhibitor, IκB.Upon stimulation with specific inducers, IκB is phosphorylated and degraded through proteosome-mediated pathways. The activated NF-κB then translocates into the nucleus and binds to specific 10 bp response elements of the IL-12, IL-23, and IL-27 genes [88, 89] Activation of NF-κB is a complex process involving the successive action of proximal NF-κB-inducing kinase (NIK) and the IκB kinases, IKKα, IKKβ, and IKKγ [90]. The expression of the IL-12 p40 subunit is controlled by proximal cis-acting elements (NF-κB half site) interacting with NF-κB family members [91]. Inhibitors of IL-12 gene expression, including retinoids, acetyl salicylic acid, and

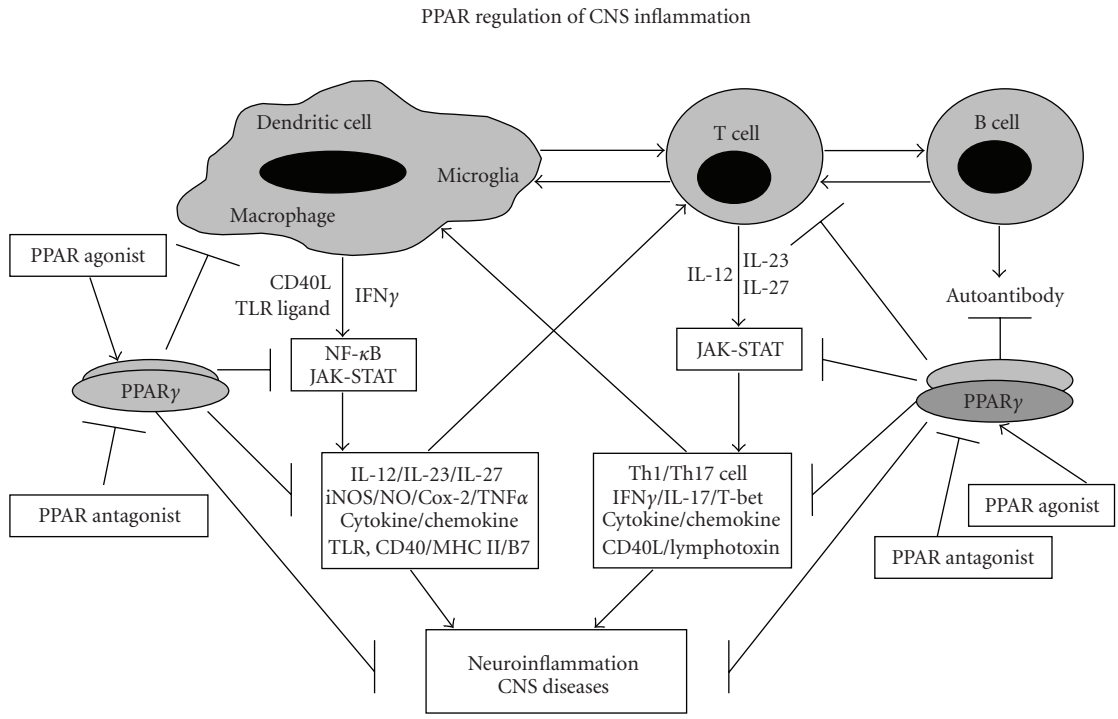

Figure 2: Regulation of neuroinflammation by PPAR agonists in CNS diseases. CD40/TLR induce the activation of NF-κB pathway leading to expression of IL-12 family cytokines from APCs which in turn signal through Jak-Stat pathway in T cells leading to Th1/Th17 differentiation and development of CNS diseases. PPAR agonists modulate signaling and transcription in APC and T cells thereby preventing CNS diseases.

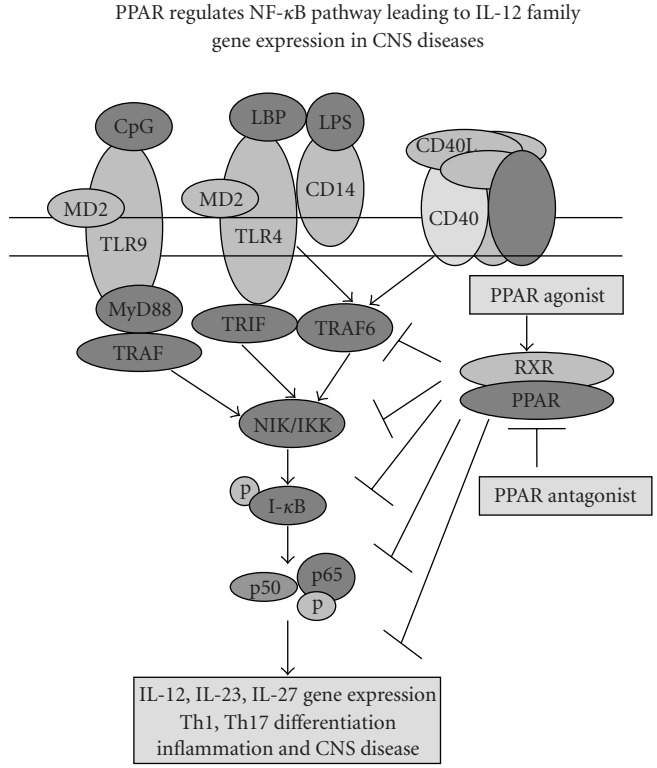

Figure 3: Regulation of NF-κB pathway by PPAR agonists in CNS diseases. The activation of microglia, macrophage and dendritic cells through toll-like receptor, CD40 or cytokine associated NF-κB pathway leads to secretion of inflammatory cytokines leading to pathogenesis of CNS diseases. PPAR agonists inhibit NF-κB pathway resulting in inhibition of CNS diseases.

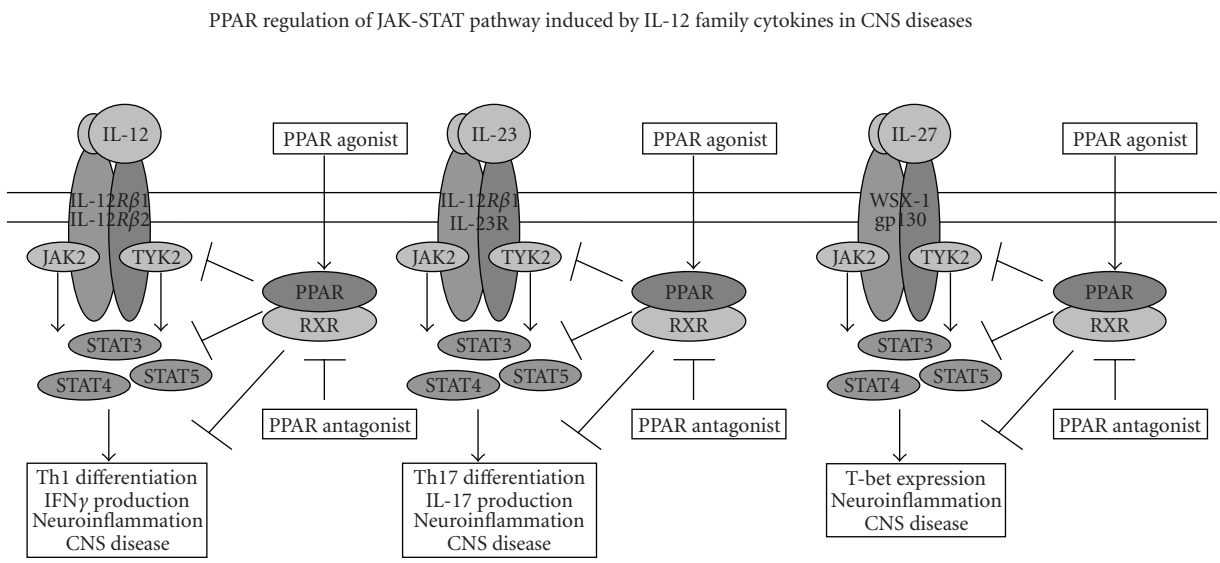

Figure 4: IL-12, IL-23 and IL-27 are heterodimeric cytokines signals through Jak-Stat pathway and induce Th1/Th17 differentiation and T-bet expression in T cells. Treatment with PPAR agonists regulate these responses in T cells resulting in inhibition of CNS inflammation.

1,25 dihydroxyvitamin D3, block NF-κB activation and bind within the IL-12p40 promoter [92, 93]. The inhibition of NF-κB pathway leading to the expression of IL-12 family cytokines by PPAR agonists suggests this be a mechanism by which PPAR agonists regulate CNS diseases (Figure 3).

The NF-κB family of proteins (RelA/p65, RelB, c-Rel, p50, p52) are widely expressed in the CNS [94] and activated in a number of CNS inflammatory diseases. Microglia plays a pivotal role in immune surveillance and host defense against infectious agents in the CNS. NF-κB, JNK, and p38 pathways are responsible for F-actin architecture during microglial activation. In AD, NF-κB activation is increased when compared to control brain. The brain samples from PD patients showed an increased nuclear p65 (RelA) in dopaminergic neurons when compared to age matched controls [95]. The spinal cord samples from ALS patients with degenerating motor neurons showed increased NF-κB activation in astrocytes that are controlled by c-jun, and JNK/SAPK kinases [96]. In MS patients, NF-κB and c-jun activities are increased in chronic lesions. PPARs are expressed in microglial cells and PPARγ agonists act as negative regulators for elements that contain Stat binding sites. While the inflammatory cascade is mediated via both NF-κB and JNK pathways, PPARγ agonists increase the levels of IκB-α and IκB-β and reduce the nuclear translocation of NF-κB [97]. While the induction of NF-κB promotes postischemic inflammation, PPAR agonists prevent postischemic inflammation and neuronal damage by inhibiting NF-κB pathway. Further analyses indicate that L-796,449 inhibits NF-κB signaling through both PPARγ-dependent and independent pathways. In addition, spinal cord injury (SCI) associated neuronal damage was less severe in NF-κB knockout mice. PPARγ induces transrepression of NF-κB-induced inflammatory genes through their association with corepressor complexes [96].

8. PPAR AGONISTS REGULATE JAK-STAT SIGNALING PATHWAY IN CNS DISEASES

The orchestrated interaction of APCs and T cells in the CNS leads to activation of Jak-Stat signaling pathway, secretion of inflammatory cytokines, and pathogenesis of neuroinflammatory diseases. The antigen-induced proliferation of T cells is a two-step process in which signaling through T cell receptor (signal 1) drives T cells from resting G0 to activated G1 phase of the cell cycle, whereas signaling through IL-2 or IL-12 receptor (second signal) is required for T cells to transit from G1 to S/G2/M phase of the cell cycle (proliferation). IL-12 is a potent inducer of G1 to S/G2/M phase transition and differentiation of Th1 cells that are critical in the pathogenesis of EAE and other CNS diseases. IL-12 signals through IL-12 receptor β1 and β2, members of the gp130 cytokine receptor super-family, expressed primarily on activated NK cells and T cells. Coexpression of IL-12Rβ1 and β2 leads to the formation of high affinity IL-12 receptors [87]. Signaling through its receptor, IL-12 induces tyrosine phosphorylation and activation of Jak2, Tyk2, Stat3, and Stat4 in T and NK cells [98, 99]. Activation of the Jak-Stat pathway leads to transcription of IL-12 response genes associated with proliferation, Th1 differentiation, and IFNγ production. IL-23 receptor is composed of common IL-12Rβ1 and a specific IL-23 receptor subunit [100]. Signaling through its receptor, IL-23 induces the activation of Jak2, Tyk2, Stat1, Stat3, Stat4, and Stat5 in T cells [98]. Activation of the Jak-Stat pathway leads to transcription of IL-23 response genes, including IL-17, which are associated with proliferation of memory T cells [101], whereas IL-27 and IFNγ activate a specific Jak-Stat pathway in T cells, resulting in the induction of T-bet in naive T cells [102]. Modulation of cytokine signaling by targeting protein tyrosine kinases or transcription factors has been considered a novel strategy

for the treatment of autoimmune diseases [103, 104]. We have shown earlier that the blockade of IL-12 signaling through Jak-Stat pathway by treatment with a Jak-2 inhibitor, tyrphostin AG490, quercetin, vitamin D, and curcumin inhibits Th1 differentiation and pathogenesis of EAE [105–108]. We have also shown recently that PPARγ agonists inhibit IL-12-induced tyrosine phosphorylation of Jak2, Tyk2, Stat3, and Stat4 in T cells, differentiation of Th1 cells and pathogenesis of EAE [30]. These findings suggest that IL-12 signaling through the Jak-Stat pathway is a molecular target in the regulation of autoimmune diseases. Recent studies have shown that the transcription factors such as Stat4 and T-bet are involved in the pathogenesis of EAE/MS, whereas Stat6 mediates recovery. While the induction of Stat1 and Stat3 promotes postischemic inflammation, and Stat-1 knockout mice develop less severe stroke lesions in the CNS [32], activation of PPARs prevents postischemic inflammation and neuronal damage (Figure 4).

The exact mechanism by which PPAR agonists negatively regulate neuroinflammation, and in particular, the Jak-Stat signaling pathway is not known. Suppressor of cytokine signaling (SOCS) proteins are negative regulators of Jak-Stat pathway. While PPARγ agonists inhibit Jak-Stat pathway in astrocytes and microglial cells, they rapidly induce the expression of SOCS 1 and 3, which in turn inhibit Jak activity in glial cells [109]. In addition, PPAR agonist can modulate Jak-Stat pathway through activation of Src homology 2 domain-containing protein phosphatase 2 (SHP2) in immune cells, thereby inhibiting neuroinflammatory diseases.

9. CONCLUSION

The neuroinflammatory diseases such as multiple sclerosis, Alzhimer's disease, stroke, and trauma are common health problems affecting more than five percent of the population worldwide. While the exact mechanisms are not known, the immune cell activation and secretion of inflammatory cytokines, involving NF-κB and Jak-Stat signaling pathways, play critical roles in the pathogenesis of many CNS diseases. Thus, interfering with the signaling network could be an effective approach in the treatment of MS and other neuroinflammatory diseases. PPAR is a family of nuclear receptor transcription factors that regulate CNS diseases by modulating neuroinflammatory signaling network. Since PPAR agonists are already in human use, they are likely to prove useful in the treatment of MS and other neuroinflammatory diseases in the near future.

REFERENCES

[1] M. M. Mustafa, O. Ramilo, K. D. Olsen, et al., "Tumor necrosis factor in mediating experimental Haemophilus influenzae type B meningitis," *The Journal of Clinical Investigation*, vol. 84, no. 4, pp. 1253–1259, 1989.

[2] C. M. L. Maffei, L. F. Mirels, R. A. Sobel, K. V. Clemons, and D. A. Stevens, "Cytokine and inducible nitric oxide synthase mRNA expression during experimental murine cryptococcal meningoencephalitis," *Infection and Immunity*, vol. 72, no. 4, pp. 2338–2349, 2004.

[3] J. Dotis and E. Roilides, "Immunopathogenesis of central nervous system fungal infections," *Neurology India*, vol. 55, no. 3, pp. 216–220, 2007.

[4] C. Lucchinetti, W. Brück, and J. Noseworthy, "Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment," *Current Opinion in Neurology*, vol. 14, no. 3, pp. 259–269, 2001.

[5] B. Becher, I. Bechmann, and M. Greter, "Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognize the brain," *Journal of Molecular Medicine*, vol. 84, no. 7, pp. 532–543, 2006.

[6] B. Hemmer, J. J. Archelos, and H.-P. Hartung, "New concepts in the immunopathogenesis of multiple sclerosis," *Nature Reviews Neuroscience*, vol. 3, no. 4, pp. 291–301, 2002.

[7] T. K. Khan and D. L. Alkon, "An internally controlled peripheral biomarker for Alzheimer's disease: Erk1 and Erk2 responses to the inflammatory signal bradykinin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 35, pp. 13203–13207, 2006.

[8] F. M. Longo and S. M. Massa, "Neuroprotective strategies in Alzheimer's disease," *Journal of the American Society of Experimental Neurotherapeutics*, vol. 1, no. 1, pp. 117–127, 2004.

[9] T. Jones, V. Ugalde, P. Franks, H. Zhou, and R. H. White, "Venous thromboembolism after spinal cord injury: incidence, time course, and associated risk factors in 16,240 adults and children," *Archives of Physical Medicine and Rehabilitation*, vol. 86, no. 12, pp. 2240–2247, 2005.

[10] T. B. Jones, R. P. Hart, and P. G. Popovich, "Molecular control of physiological and pathological T-cell recruitment after mouse spinal cord injury," *Journal of Neuroscience*, vol. 25, no. 28, pp. 6576–6583, 2005.

[11] D. M. Wingerchuk, C. F. Lucchinetti, and J. H. Noseworthy, "Multiple sclerosis: current pathophysiological concepts," *Laboratory Investigation*, vol. 81, no. 3, pp. 263–281, 2001.

[12] B. Blumberg and R. M. Evans, "Orphan nuclear receptors—new ligands and new possibilities," *Genes & Development*, vol. 12, no. 20, pp. 3149–3155, 1998.

[13] J. Uppenberg, C. Svensson, M. Jaki, G. Bertilsson, L. Jendeberg, and A. Berkenstam, "Crystal structure of the ligand binding domain of the human nuclear receptor PPARγ," *The Journal of Biological Chemistry*, vol. 273, no. 47, pp. 31108–31112, 1998.

[14] D. Shao, S. M. Rangwala, S. T. Bailey, S. L. Krakow, M. J. Reginato, and M. A. Lazar, "Interdomain communication regulating ligand binding by PPAR-γ," *Nature*, vol. 396, no. 6709, pp. 377–380, 1998.

[15] S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans, "Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors," *Nature*, vol. 358, no. 6389, pp. 771–774, 1992.

[16] R. T. Nolte, G. B. Wisely, S. Westin, et al., "Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ," *Nature*, vol. 395, no. 6698, pp. 137–143, 1998.

[17] R. Janknecht and T. Hunter, "A growing coactivator network," *Nature*, vol. 383, no. 6595, pp. 22–23, 1996.

[18] Nuclear Receptors Nomenclature Committee, "A unified nomenclature system for the nuclear receptor superfamily," *Cell*, vol. 97, no. 2, pp. 161–163, 1999.

[19] K. Murakami, K. Tobe, T. Ide, et al., "A novel insulin sensitizer acts as a coligand for peroxisome proliferator-activated receptor-α (PPAR-α) and PPAR-γ. Effect of PPAR-α

activation on abnormal lipid metabolism in liver of Zucker fatty rats," *Diabetes*, vol. 47, no. 12, pp. 1841–1847, 1998.

[20] G. Krey, O. Braissant, F. L'Horset, et al., "Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay," *Molecular Endocrinology*, vol. 11, no. 6, pp. 779–791, 1997.

[21] J. M. Peters, S. S. T. Lee, W. Li, et al., "Growths, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor $\beta(\delta)$," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[22] I. Saluja, J. G. Granneman, and R. P. Skoff, "PPAR δ agonists stimulate oligodendrocyte differentiation in tissue culture," *Glia*, vol. 33, no. 3, pp. 191–204, 2001.

[23] P. E. Polak, S. Kalinin, C. Dello Russo, et al., "Protective effects of a peroxisome proliferator-activated receptor-β/δ agonist in experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 168, no. 1-2, pp. 65–75, 2005.

[24] J.-B. Pialat, T.-H. Cho, O. Beuf, et al., "MRI monitoring of focal cerebral ischemia in peroxisome proliferator-activated receptor (PPAR)-deficient mice," *NMR in Biomedicine*, vol. 20, no. 3, pp. 335–342, 2007.

[25] W. W. Harrington, C. S. Britt, J. G. Wilson, et al., "The effect of PPARα, PPARδ, PPARγ, and PPARpan agonists on body weight, body mass, and serum lipid profiles in diet-induced obese AKR/J Mice," *PPAR Research*, vol. 2007, Article ID 97125, 13 pages, 2007.

[26] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[27] P. D. Storer, J. Xu, J. Chavis, and P. D. Drew, "Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroimmunology*, vol. 161, no. 1-2, pp. 113–122, 2005.

[28] P. D. Storer, J. Xu, J. A. Chavis, and P. D. Drew, "Cyclopentenone prostaglandins PGA_2 and 15-deoxy-$\Delta^{12,14}PGJ_2$ suppress activation of marine microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroscience Research*, vol. 80, no. 1, pp. 66–74, 2005.

[29] D. Dey, S. Medicherla, P. Neogi, et al., "A novel peroxisome proliferator-activated gamma (PPARγ) agonist, CLX-0921, has potent antihyperglycemic activity with low adipogenic potential," *Metabolism*, vol. 52, no. 8, pp. 1012–1018, 2003.

[30] C. Natarajan and J. J. Bright, "Peroxisome proliferator-activated receptor-gamma agonist inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation," *Genes & Immunity*, vol. 3, no. 2, pp. 59–70, 2002.

[31] A. Diab, C. Deng, J. D. Smith, et al., "Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis," *The Journal of Immunology*, vol. 168, no. 5, pp. 2508–2515, 2002.

[32] D. L. Feinstein, E. Galea, V. Gavrilyuk, et al., "Peroxisome proliferator-activated receptor-γ agonists prevent experimental autoimmune encephalomyelitis," *Annals of Neurology*, vol. 51, no. 6, pp. 694–702, 2002.

[33] P. D. Storer, J. Xu, J. Chavis, and P. D. Drew, "Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroimmunology*, vol. 161, no. 1-2, pp. 113–122, 2005.

[34] M. Niino, K. Iwabuchi, S. Kikuchi, et al., "Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-γ," *Journal of Neuroimmunology*, vol. 116, no. 1, pp. 40–48, 2001.

[35] S. Schmidt, E. Moric, M. Schmidt, M. Sastre, D. L. Feinstein, and M. T. Heneka, "Anti-inflammatory and antiproliferative actions of PPAR-γ agonists on T lymphocytes derived from MS patients," *Journal of Leukocyte Biology*, vol. 75, no. 3, pp. 478–485, 2004.

[36] L. Klotz, M. Schmidt, T. Giese, et al., "Proinflammatory stimulation and pioglitazone treatment regulate peroxisome proliferator-activated receptor γ levels in peripheral blood mononuclear cells from healthy controls and multiple sclerosis patients," *The Journal of Immunology*, vol. 175, no. 8, pp. 4948–4955, 2005.

[37] J. J. Bright, C. Natarajan, G. Muthian, Y. Barak, and R. M. Evans, "Peroxisome proliferator-activated receptor-γ-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis," *The Journal of Immunology*, vol. 171, no. 11, pp. 5743–5750, 2003.

[38] H. P. Raikwar, G. Muthian, J. Rajasingh, C. N. Johnson, and J. J. Bright, "PPARγ antagonists reverse the inhibition of neural antigen-specific Th_1 response and experimental allergic encephalomyelitis by Ciglitazone and 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2," *Journal of Neuroimmunology*, vol. 178, no. 1-2, pp. 76–86, 2006.

[39] P. B. Rosenberg, "Clinical aspects of inflammation in Alzheimer's disease," *International Review of Psychiatry*, vol. 17, no. 6, pp. 503–514, 2005.

[40] M. Hirohata, K. Ono, H. Naiki, and M. Yamada, "Non-steroidal anti-inflammatory drugs have anti-amyloidogenic effects for Alzheimer's β-amyloid fibrils in vitro," *Neuropharmacology*, vol. 49, no. 7, pp. 1088–1099, 2005.

[41] D. M. McTigue, R. Tripathi, P. Wei, and A. T. Lash, "The PPAR gamma agonist Pioglitazone improves anatomical and locomotor recovery after rodent spinal cord injury," *Experimental Neurology*, vol. 205, no. 2, pp. 396–406, 2007.

[42] M. T. Heneka, M. Sastre, L. Dumitrescu-Ozimek, et al., "Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV717I transgenic mice," *Brain*, vol. 128, no. 6, pp. 1442–1453, 2005.

[43] G. S. Watson, B. A. Cholerton, M. A. Reger, et al., "Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study," *American Journal of Geriatric Psychiatry*, vol. 13, no. 11, pp. 950–958, 2005.

[44] A. D. Wahner, J. M. Bronstein, Y. M. Bordelon, and B. Ritz, "Nonsteroidal anti-inflammatory drugs may protect against Parkinson disease," *Neurology*, vol. 69, no. 19, pp. 1836–1842, 2007.

[45] D. Casper, U. Yaparpalvi, N. Rempel, and P. Werner, "Ibuprofen protects dopaminergic neurons against glutamate toxicity in vitro," *Neuroscience Letters*, vol. 289, no. 3, pp. 201–204, 2000.

[46] A. D. Wahner, J. M. Bronstein, Y. M. Bordelon, and B. Ritz, "Nonsteroidal anti-inflammatory drugs may protect against Parkinson disease," *Neurology*, vol. 69, no. 19, pp. 1836–1842, 2007.

[47] D. Dobrian, S. D. Schriver, A. A. Khraibi, and R. L. Prewitt, "Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity," *Hypertension*, vol. 43, no. 1, pp. 18–56, 2004.

[48] B. Schütz, J. Reimann, L. Dumitrescu-Ozimek, et al., "The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice," *Journal of Neuroscience*, vol. 25, no. 34, pp. 7805–7812, 2005.

[49] D. Deplanque, "Cell protection through PPAR nuclear receptor activation," *Thérapie*, vol. 59, no. 1, pp. 25–29, 2004.

[50] J. Culman, Y. Zhao, P. Gohlke, and T. Herdegen, "PPAR-γ: therapeutic target for ischemic stroke," *Trends in Pharmacological Sciences*, vol. 28, no. 5, pp. 244–249, 2007.

[51] S. T. Fujimoto, L. Longhi, K. E. Saatman, and T. K. McIntosh, "Motor and cognitive function evaluation following experimental traumatic brain injury," *Neuroscience & Biobehavioral Reviews*, vol. 28, no. 4, pp. 365–378, 2004.

[52] B.-C. Lee, H.-K. Doo, S.-Y. Ahn, et al., "Peroxisome proliferator-activated receptor-γ Pro12Ala polymorphism is associated with the susceptibility to ischemic stroke in Taeeumin classified by Sasang medicine," *Neurological Research*, vol. 29, supplement 1, pp. 32–37, 2007.

[53] M. Allahtavakoli, A. P. Shabanzadeh, S. S. Sadr, M. Parviz, and B. Djahanguiri, "Rosiglitazone, a peroxisome proliferator-activated receptor-γ ligand, reduces infarction volume and neurological deficits in an embolic model of stroke," *Clinical and Experimental Pharmacology and Physiology*, vol. 33, no. 11, pp. 1052–1058, 2006.

[54] Z. Ou, X. Zhao, L. A. Labiche, et al., "Neuronal expression of peroxisome proliferator-activated receptor-gamma (PPARγ) and 15d-prostaglandin J_2—mediated protection of brain after experimental cerebral ischemia in rat," *Brain Research*, vol. 1096, no. 1, pp. 196–203, 2006.

[55] Y. Zhao, A. Patzer, P. Gohlke, T. Herdegen, and J. Culman, "The intracerebral application of the PPARγ-ligand pioglitazone confers neuroprotection against focal ischaemia in the rat brain," *European Journal of Neuroscience*, vol. 22, no. 1, pp. 278–282, 2005.

[56] K. Tureyen, R. Kapadia, K. K. Bowen, et al., "Peroxisome proliferator-activated receptor-γ agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents," *Journal of Neurochemistry*, vol. 101, no. 1, pp. 41–56, 2007.

[57] M. P. Pereira, O. Hurtado, A. Cárdenas, et al., "The nonthiazolidinedione PPARγ agonist L-796,449 is neuroprotective in experimental stroke," *Journal of Neuropathology & Experimental Neurology*, vol. 64, no. 9, pp. 797–805, 2005.

[58] T. Nakamura, E. Yamamoto, K. Kataoka, et al., "Pioglitazone exerts protective effects against stroke in stroke-prone spontaneously hypertensive rats, independently of blood pressure," *Stroke*, vol. 38, no. 11, pp. 3016–3022, 2007.

[59] A. M. Lincoff, K. Wolski, S. J. Nicholls, and S. E. Nissen, "Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials," *The Journal of the American Medical Association*, vol. 298, no. 10, pp. 1180–1188, 2007.

[60] S. Dasgupta, A. Roy, M. Jana, D. M. Hartley, and K. Pahan, "Gemfibrozil ameliorates relapsing-remitting experimental autoimmune encephalomyelitis independent of peroxisome proliferator-activated receptor-α," *Molecular Pharmacology*, vol. 72, no. 4, pp. 934–946, 2007.

[61] D. Deplanque, P. Gelé, O. Pétrault, et al., "Peroxisome proliferator-activated receptor-α activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment," *The Journal of Neuroscience*, vol. 23, no. 15, pp. 6264–6271, 2003.

[62] M. Collino, M. Aragno, R. Mastrocola, et al., "Oxidative stress and inflammatory response evoked by transient cerebral ischemia/reperfusion: effects of the PPAR-α agonist WY14643," *Free Radical Biology and Medicine*, vol. 41, no. 4, pp. 579–589, 2006.

[63] V. C. Besson, X. R. Chen, M. Plotkine, and C. Marchand-Verrecchia, "Fenofibrate, a peroxisome proliferator-activated receptor α agonist, exerts neuroprotective effects in traumatic brain injury," *Neuroscience Letters*, vol. 388, no. 1, pp. 7–12, 2005.

[64] Ş. Gül, S. E. Çelik, M. Kalayci, M. Taşyürekli, N. Çokar, and T. Bilge, "Dose-dependent neuroprotective effects of melatonin on experimental spinal cord injury in rats," *Surgical Neurology*, vol. 64, no. 4, pp. 355–361, 2005.

[65] S. S. Haghighi, S. K. Agrawal, D. Surdell Jr., et al., "Effects of methylprednisolone and MK-801 on functional recovery after experimental chronic spinal cord injury," *Spinal Cord*, vol. 38, no. 12, pp. 733–740, 2000.

[66] P. E. Polak, S. Kalinin, C. Dello Russo, et al., "Protective effects of a peroxisome proliferator-activated receptor-β/δ agonist in experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 168, no. 1-2, pp. 65–75, 2005.

[67] A. Iwashita, Y. Muramatsu, T. Yamazaki, et al., "Neuroprotective efficacy of the peroxisome proliferator-activated receptor δ-selective agonists in vitro and in vivo," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1087–1096, 2007.

[68] J. P. Leonard, K. E. Waldburger, and S. J. Goldman, "Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12," *Journal of Experimental Medicine*, vol. 181, no. 1, pp. 381–386, 1995.

[69] J. J. Bright, B. F. Musuro, C. Du, and S. Sriram, "Expression of IL-12 in CNS and lymphoid organs of mice with experimental allergic encephalitis," *Journal of Neuroimmunology*, vol. 82, no. 1, pp. 22–30, 1998.

[70] D. J. Cua, J. Sherlock, Y. Chen, et al., "Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain," *Nature*, vol. 421, no. 6924, pp. 744–748, 2003.

[71] A. Szczuciński and J. Losy, "Chemokines and chemokine receptors in multiple sclerosis. Potential targets for new therapies," *Acta Neurologica Scandinavica*, vol. 115, no. 3, pp. 137–146, 2007.

[72] E. Sindern, "Role of chemokines and their receptors in the pathogenesis of multiple sclerosis," *Frontiers in Bioscience*, vol. 9, pp. 457–463, 2004.

[73] A. R. Glabinski and R. M. Ransohoff, "Targeting the chemokine system for multiple sclerosis treatment," *Current Opinion in Investigational Drugs*, vol. 2, no. 12, pp. 1712–1719, 2001.

[74] D. M. Muller, M. P. Pender, and J. M. Greer, "Chemokines and chemokine receptors: potential therapeutic targets in multiple sclerosis," *Current Drug Targets: Inflammation & Allergy*, vol. 3, no. 3, pp. 279–290, 2004.

[75] M. Ukkonen, X. Wu, B. Reipert, P. Dastidar, and I. Elovaara, "Cell surface adhesion molecules and cytokine profiles in

primary progressive multiple sclerosis," *Multiple Sclerosis*, vol. 13, no. 6, pp. 701–707, 2007.

[76] H. A. Pershadsingh, M. T. Heneka, R. Saini, N. M. Amin, D. J. Broeske, and D. L. Feinstein, "Effect of pioglitazone treatment in a patient with secondary multiple sclerosis," *Journal of Neuroinflammation*, vol. 1, article 3, pp. 1–4, 2004.

[77] S. Redondo, E. Ruiz, C. G. Santos-Gallego, E. Padilla, and T. Tejerina, "Pioglitazone induces vascular smooth muscle cell apoptosis through a peroxisome proliferator-activated receptor-γ, transforming growth factor-β1, and a Smad2-dependent mechanism," *Diabetes*, vol. 54, no. 3, pp. 811–817, 2005.

[78] E. M. Shevach, J. T. Chang, and B. M. Segal, "The critical role of IL-12 and the IL-12Rβ2 subunit in the generation of pathogenic autoreactive Th1 cells," *Springer Seminars in Immunopathology*, vol. 21, no. 3, pp. 249–262, 1999.

[79] B. M. Segal, B. K. Dwyer, and E. M. Shevach, "An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease," *Journal of Experimental Medicine*, vol. 187, no. 4, pp. 537–546, 1998.

[80] W. T. Watford, B. D. Hissong, J. H. Bream, Y. Kanno, L. Muul, and J. J. O'Shea, "Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4," *Immunological Reviews*, vol. 202, no. 1, pp. 139–156, 2004.

[81] T. Touil, D. Fitzgerald, G.-X. Zhang, A. M. Rostami, and B. Gran, "Pathophysiology of interleukin-23 in experimental autoimmune encephalomyelitis," *Drug News & Perspectives*, vol. 19, no. 2, pp. 77–83, 2006.

[82] M. Veldhoen, R. J. Hocking, C. J. Atkins, R. M. Locksley, and B. Stockinger, "TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells," *Immunity*, vol. 24, no. 2, pp. 179–189, 2006.

[83] S. Kamiya, T. Owaki, N. Morishima, F. Fukai, J. Mizuguchi, and T. Yoshimoto, "An indispensable role for STAT1 in IL-27-induced T-bet expression but not proliferation of naive $CD4^+$ T cells," *The Journal of Immunology*, vol. 173, no. 6, pp. 3871–3877, 2004.

[84] A. R. Gocke, P. D. Cravens, L.-H. Ben, et al., "T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity," *The Journal of Immunology*, vol. 178, no. 3, pp. 1341–1348, 2007.

[85] L. W. Collison, C. J. Workman, T. T. Kuo, et al., "The inhibitory cytokine IL-35 contributes to regulatory T-cell function," *Nature*, vol. 450, no. 7169, pp. 566–569, 2007.

[86] E. A. Wohlfert, F. C. Nichols, E. Nevius, and R. B. Clark, "Peroxisome proliferator-activated receptor γ (PPARγ) and immunoregulation: enhancement of regulatory T cells through PPARγ-dependent and -independent mechanisms," *The Journal of Immunology*, vol. 178, no. 7, pp. 4129–4135, 2007.

[87] G. Trinchieri, S. Pflanz, and R. A. Kastelein, "The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses," *Immunity*, vol. 19, no. 5, pp. 641–644, 2003.

[88] S. Ghosh, M. J. May, and E. B. Kopp, "NF-κB and rel proteins: evolutionarily conserved mediators of immune responses," *Annual Review of Immunology*, vol. 16, pp. 225–260, 1998.

[89] W. C. Sha, "Regulation of immune responses by NF-κB/Rel transcription factors," *Journal of Experimental Medicine*, vol. 187, no. 2, pp. 143–146, 1998, erratum in *Journal of Experimental Medicine*, vol. 187, no. 4, p. 661, 1998.

[90] J. D. Woronicz, X. Gao, Z. Cao, M. Rothe, and D. V. Goeddel, "IκB kinase-β: NF-κB activation and complex formation with IκB kinase-α and NIK," *Science*, vol. 278, no. 5339, pp. 866–869, 1997.

[91] T. L. Murphy, M. G. Cleveland, P. Kulesza, J. Magram, and K. M. Murphy, "Regulation of interleukin 12 p40 expression through an NF-κB half-site," *Molecular and Cellular Biology*, vol. 15, no. 10, pp. 5258–5267, 1995.

[92] D. Mazzeo, P. Panina-Bordignon, H. Recalde, F. Sinigaglia, and D. D'Ambrosio, "Decreased IL-12 production and Th1 cell development by acetyl salicylic acid-mediated inhibition of NF-κB," *European Journal of Immunology*, vol. 28, no. 10, pp. 3205–3213, 1998.

[93] D. D'Ambrosio, M. Cippitelli, M. G. Cocciolo, et al., "Inhibition of IL-12 production by 1,25-dihydroxyvitamin D_3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene," *The Journal of Clinical Investigation*, vol. 101, no. 1, pp. 252–262, 1998.

[94] M. Grilli and M. Memo, "Nuclear factor-κB/Rel proteins: a point of convergence of signalling pathways relevant in neuronal function and dysfunction," *Biochemical Pharmacology*, vol. 57, no. 1, pp. 1–7, 1999.

[95] M. P. Mattson and S. Camandola, "NF-κB in neuronal plasticity and neurodegenerative disorders," *The Journal of Clinical Investigation*, vol. 107, no. 3, pp. 247–254, 2001.

[96] A. Migheli, R. Piva, C. Atzori, D. Troost, and D. Schiffer, "c-Jun, JNK/SAPK kinases and transcription factor NF-κB are selectively activated in astrocytes, but not motor neurons, in amyotrophic lateral sclerosis," *Journal of Neuropathology & Experimental Neurology*, vol. 56, no. 12, pp. 1314–1322, 1997.

[97] T. Dehmer, M. T. Heneka, M. Sastre, J. Dichgans, and J. B. Schulz, "Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with IκBα induction and block of NFκB and iNOS activation," *Journal of Neurochemistry*, vol. 88, no. 2, pp. 494–501, 2004.

[98] N. G. Jacobson, S. J. Szabo, R. M. Weber-Nordt, et al., "Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4," *Journal of Experimental Medicine*, vol. 181, no. 5, pp. 1755–1762, 1995.

[99] C. M. Bacon, E. F. Petricoin III, J. R. Ortaldo, et al., "Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 16, pp. 7307–7311, 1995.

[100] C. Parham, M. Chirica, J. Timans, et al., "A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R," *The Journal of Immunology*, vol. 168, no. 11, pp. 5699–5708, 2002.

[101] S. Aggarwal, N. Ghilardi, M.-H. Xie, F. J. de Sauvage, and A. L. Gurney, "Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17," *The Journal of Biological Chemistry*, vol. 278, no. 3, pp. 1910–1914, 2003.

[102] P. J. Murray, "The JAK-STAT signaling pathway: Input and output integration," *The Journal of Immunology*, vol. 178, no. 5, pp. 2623–2629, 2007.

[103] J. J. O'Shea, H. Park, M. Pesu, D. Borie, and P. Changelian, "New strategies for immunosuppression: interfering with cytokines by targeting the Jak/Stat pathway," *Current Opinion in Rheumatology*, vol. 17, no. 3, pp. 305–311, 2005.

[104] H. M. Seidel, P. Lamb, and J. Rosen, "Pharmaceutical intervention in the JAK/STAT signaling pathway," *Oncogene*, vol. 19, no. 21, pp. 2645–2656, 2000.

[105] J. J. Bright, D. Du, and S. Sriram, "Tyrphostin B42 inhibits IL-12-induced tyrosine phosphorylation and activation of Janus kinase-2 and prevents experimental allergic encephalomyelitis," *The Journal of Immunology*, vol. 162, no. 10, pp. 6255–6262, 1999.

[106] G. Muthian and J. J. Bright, "Quercetin, a flavonoid phytoestrogen, ameliorates experimental allergic encephalomyelitis by blocking IL-12 signaling through JAK-STAT pathway in T lymphocyte," *Journal of Clinical Immunology*, vol. 24, no. 5, pp. 542–552, 2004.

[107] G. Muthian, H. P. Raikwar, J. Rajasingh, and J. J. Bright, "1,25 dihydroxyvitamin-D3 modulates JAK-STAT pathway in IL-12/IFNγ axis leading to Th1 response in experimental allergic encephalomyelitis," *Journal of Neuroscience Research*, vol. 83, no. 7, pp. 1299–1309, 2006.

[108] C. Natarajan and J. J. Bright, "Curcumin inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through Janus kinase-STAT pathway in T lymphocytes," *The Journal of Immunology*, vol. 168, no. 12, pp. 6506–6513, 2002.

[109] T. L. Lee, J. Yeh, C. Van Waes, and Z. Chen, "Epigenetic modification of *SOCS-1* differentially regulates STAT3 activation in response to interleukin-6 receptor and epidermal growth factor receptor signaling through JAK and/or MEK in head and neck squamous cell carcinomas," *Molecular Cancer Therapeutics*, vol. 5, no. 1, pp. 8–19, 2006.

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Research Article

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

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Microglia and astrocytes express numerous members of the Toll-like receptor (TLR) family that are pivotal for recognizing conserved microbial motifs expressed by a wide array of pathogens. Despite the critical role for TLRs in pathogen recognition, when dysregulated these pathways can also exacerbate CNS tissue destruction. Therefore, a critical balance must be achieved to elicit sufficient immunity to combat CNS infectious insults and downregulate these responses to avoid pathological tissue damage. We performed a comprehensive survey on the efficacy of various PPAR-γ agonists to modulate proinflammatory mediator release from primary microglia and astrocytes in response to numerous TLR ligands relevant to CNS infectious diseases. The results demonstrated differential abilities of select PPAR-γ agonists to modulate glial activation. For example, 15d-PGJ_2 and pioglitazone were both effective at reducing IL-12 p40 release by TLR ligand-activated glia, whereas CXCL2 expression was either augmented or inhibited by 15d-PGJ_2, effects that were dependent on the TLR ligand examined. Pioglitazone and troglitazone demonstrated opposing actions on microglial CCL2 production that were TLR ligand-dependent. Collectively, this information may be exploited to modulate the host immune response during CNS infections to maximize host immunity while minimizing inappropriate bystander tissue damage that is often characteristic of such diseases.

1. INTRODUCTION

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

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

of neuroinflammation is paramount and under certain conditions inappropriate TLR activation may contribute to excessive tissue destruction. Therefore, modulating TLR activity to achieve optimal benefit for the host may be a plausible strategy for minimizing tissue damage during neuroinflammatory disorders.

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

2. MATERIALS AND METHODS

2.1. TLR ligands and PPAR-γ agonists

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

The natural PPAR-γ agonist 15d-PGJ_2 and synthetic TZDs ciglitazone, rosiglitazone, pioglitazone, and troglitazone were purchased from Cayman Chemical (see Table 2; PPAR-γ Pak; Ann Arbor , Mich, USA). Dose-response studies were performed for all TZDs (10, 30, and 100 μM) and 15d-PGJ_2 (5, 10, and 20 μM) in both TLR-activated microglia and astrocytes.

2.2. Primary microglia and astrocyte cultures

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

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

2.3. Cell viability assays

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

2.4. Enzyme-linked immunosorbent assays (ELISAs)

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

2.5. Nitrite assay

Nitrite levels, a stable end product resulting from the reaction of NO with molecular oxygen, were determined in astrocytes by adding 50 μl of cell-conditioned culture medium with 50 μl of Griess reagent (0.1% naphtyletylenediamine dihydrochloride, 1% sulfanilamide, 2.5% phosphoric acid, all from Sigma) in a 96-well plate. The absorbance at 550 nm was measured on a plate reader (Spectra Max 190, Molecular Devices, Sunnyvale , Calif , USA), and nitrite concentrations were calculated using a standard curve with sodium nitrite ($NaNO_2$, Sigma, level of sensitivity, 0.4 μM). Based on our previous findings where *S. aureus*-derived TLR ligands were

Table 1: Summary of PPAR-γ effects on PAMP-activated microglia reported in this study.

	TLR ligand					
PPAR-γ agonist	Pam3Cys	polyI:C	LPS	Flagellin	ssRNA	ODN
15d-PGJ_2	↓ IL-12 ↓ TNF-α ↓ CXCL2 ↓ CCL2	↓ IL-12 ↓ TNF-α ↑ CXCL2 ↓ CCL2	↓ IL-12 ↓ TNF-α ↑ CXCL2 ↓ CCL2	↓ IL-12 ↓ TNF-α No effect CXCL2 ↓ CCL2	↓ IL-12 TNF-α ND* No effect CXCL2 No effect CCL2	↓ IL-12 ↓ TNF-α ↑ CXCL2 ↓ CCL2
Rosiglitazone	↓ IL-12 ↓ TNF-α	↓ IL-12 ↓ TNF-α	↓ IL-12 No effect TNF-α	↓ IL-12 ↓ TNF-α	↓ IL-12 TNF-α ND	↓ IL-12 No effect TNF-α
Pioglitazone	↓ IL-12 ↑ CXCL2	↓ IL-12	↓ IL-12	↓ IL-12	↓ IL-12	↓ IL-12 ↑ CXCL2

*ND; not detectable.

Table 2: Summary of PPAR-γ effects on PAMP-activated astrocytes reported in this study.

	TLR ligand					
PPAR-γ agonist	Pam3Cys	polyI:C	LPS	Flagellin	ssRNA	ODN
15d-PGJ_2	↓ IL-12 ↑ CXCL2	↓ IL-12 ↓ NO	↓ IL-12 ↓ NO ↑ CXCL2	↓ IL-12	↓ IL-12	↓ IL-12
Ciglitazone	↓ CXCL2	↓ CXCL2	↓ CXCL2	↓ CXCL2	NR*	↓ CXCL2
Pioglitazone	↓ IL-12 ↑ CXCL2	↓ IL-12	↓ IL-12	↓ IL-12 ↑ CXCL2 ↑ CCL2	↓ IL-12 ↑ CXCL2 ↑ CCL2	↓ IL-12
Troglitazone	↓ CCL2	↓ CCL2	NR	↓ CCL2	NR	↓ CCL2

*NR: not reported.

potent inducers of NO in astrocytes but not microglia [24, 33, 34], we only quantitated NO levels in the former in the current study.

2.6. Statistics

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

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

3. RESULTS

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

Microglia represent the main innate immune effector in the CNS parenchyma as evident by their expression of numerous TLRs [32, 37, 38]. Although much emphasis has been placed on the neurodestructive properties of activated microglia, recent studies have revealed that in the correct context microglia can also facilitate CNS repair [39–41]. Therefore, striking the correct balance between regulated and inappropriate microglial activation may lead to optimal outcomes for a wide range of CNS neuroinflammatory conditions. To determine whether PPAR-γ agonists could serve to modulate microglial activation in the context of CNS infection, the effects of these compounds on microglial cytokine production in response to diverse TLR ligands were examined. The natural PPAR-γ agonist 15d-PGJ_2 was uniformly found to inhibit IL-12 p40 release in response to all TLR ligands tested including Pam3Cys4, polyI:C, LPS, flagellin, ssRNA, and ODN (Figure 1). Fairly comprehensive reductions in IL-12 p40 production were also observed with all TLR ligands tested in response to the synthetic PPAR-γ agonists rosiglitazone (Figure 2) and pioglitazone (Figure 3) although the extent of inhibition was dramatically less compared to 15d-PGJ_2. Rosiglitazone exhibited significant toxicity to primary microglia at the highest dose tested (i.e., 100 μM), hence it was not included in the final analysis. In contrast, ciglitazone did not dramatically affect IL-12 p40 production in response to the majority of TLR ligands examined (data not shown). Troglitazone exhibited microglial toxicity at the two highest doses of agonist tested (i.e., 100 and 30 μM); therefore, the results of this PPAR-γ agonist on microglial mediator production in response to TLR ligands are not presented.

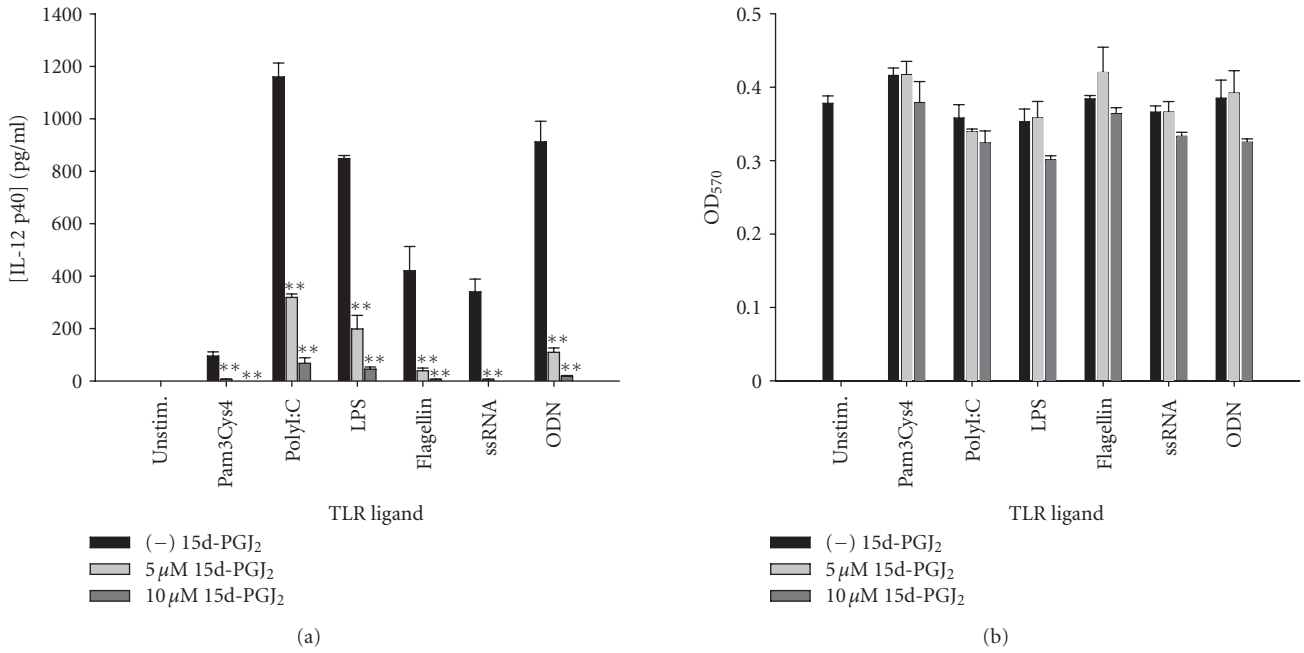

Figure 1: *The natural PPAR-γ agonist 15d-PGJ$_2$ is a potent inhibitor of microglial IL-12 p40 production in response to a vast array of TLR ligands.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of 15d-PGJ$_2$ on microglial viability were assessed using an MTT assay (b). Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks (**$P < .001$). Results presented are representative of two independent experiments.

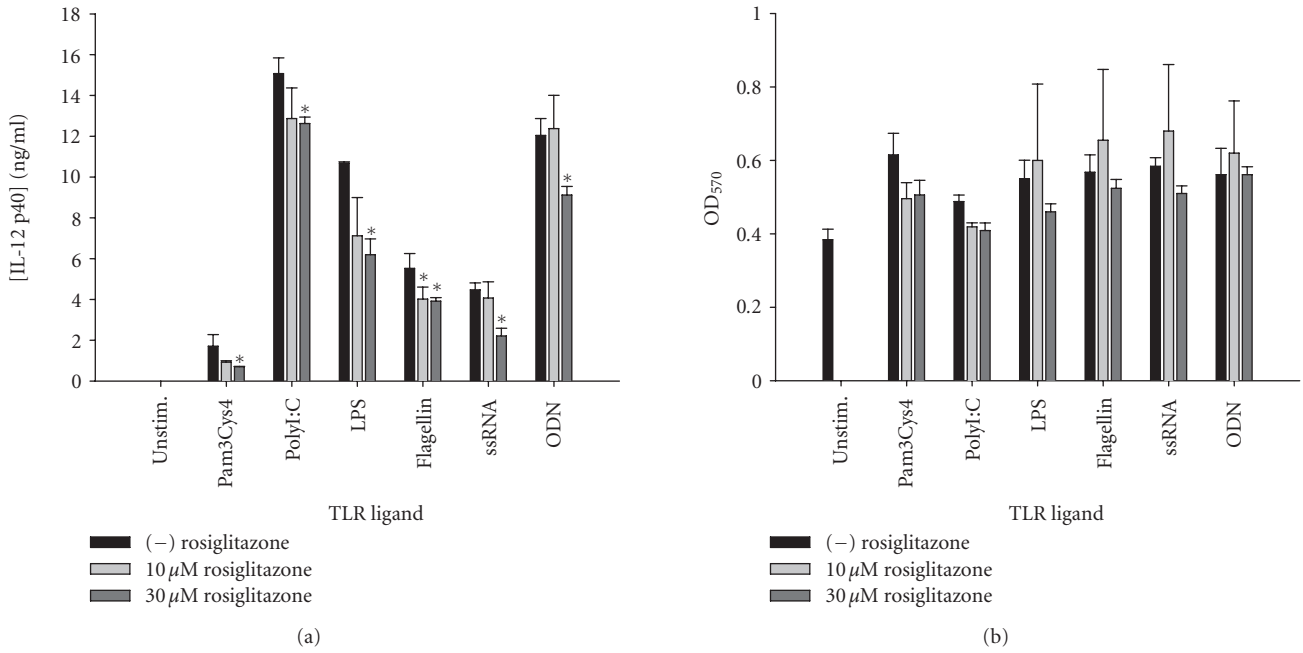

Figure 2: *The synthetic PPAR-γ agonist rosiglitazone attenuates IL-12 p40 production in response to TLR ligands in microglia.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of rosiglitazone prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of rosiglitazone on microglial viability were assessed using an MTT assay (b). Significant differences between microglia treated with TLR ligands alone versus TLR ligands + rosiglitazone are noted with asterisks (*$P < .05$). Results presented are representative of two independent experiments.

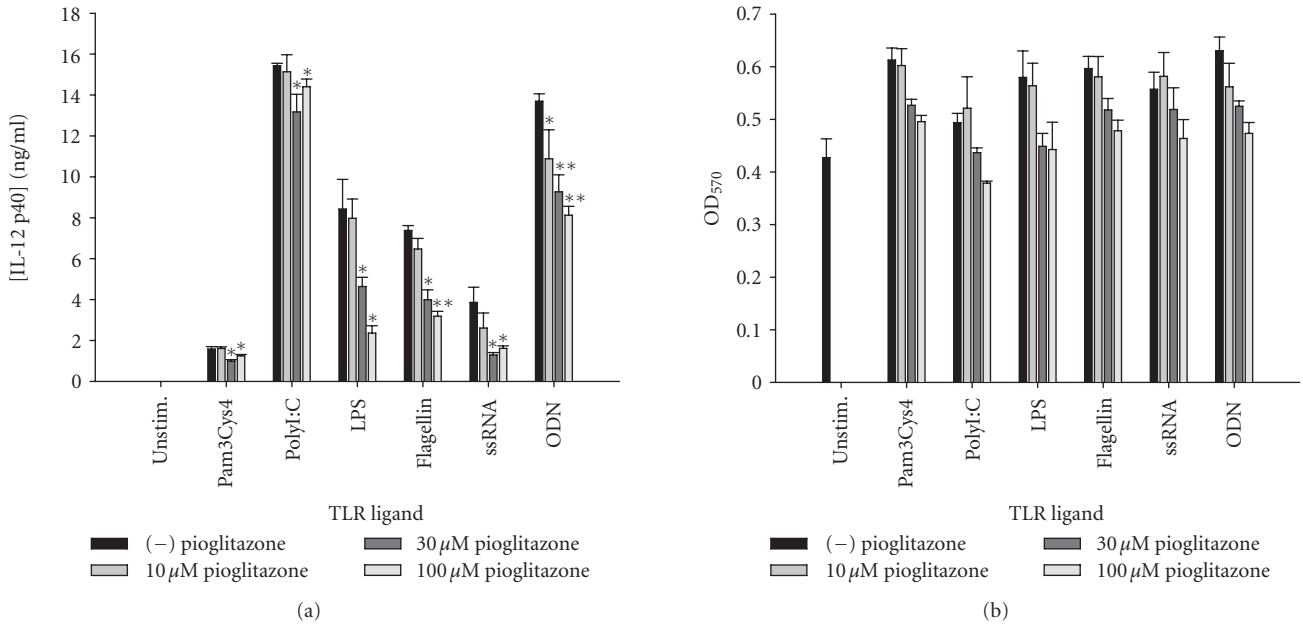

Figure 3: *The TZD pioglitazone inhibits microglial IL-12 p40 expression in response to diverse TLR ligands.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a). The effects of pioglitazone on microglial viability were assessed using an MTT assay (b). Significant differences between microglia treated with TLR ligands alone versus TLR ligands + pioglitazone are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

Another proinflammatory cytokine with potent effects on the blood-brain barrier as well as glial activation is TNF-α [42]. This cytokine is expressed at high levels in numerous CNS infectious diseases including bacterial meningitis, brain abscess, as well as viral infections [43–46]. In some cases, excessive TNF-α production during these infectious diseases has been implicated in contributing to bystander damage to surrounding host tissue [45, 46]. Therefore, strategies aimed at achieving optimized cytokine expression may prove beneficial for favorable disease outcomes. Of the PPAR-γ agonists tested, 15d-PGJ_2 was found to exert the most global inhibition of TNF-α production in response to the battery of TLR ligands tested (Figure 4). Specifically, TNF-α release by microglia in response to Pam3Cys4, polyI:C, flagellin, and ODN was significantly attenuated by 15d-PGJ_2, whereas cytokine production following LPS treatment was not as dramatically affected. Single-stranded RNA (ssRNA) was a poor inducer of TNF-α by primary microglia (Figure 4). Similar to results with IL-12 p40, rosiglitazone was the next more global inhibitor of TNF-α production in response to the TLR ligands tested (Figure 5), whereas the other PPAR-γ agonists (i.e., ciglitazone and pioglitazone) were largely without effect (data not shown). Collectively, these results indicate that not all PPAR-γ agonists are equally effective at modulating proinflammatory cytokine release from primary microglia and suggest that tailored responses to specific pathogen motifs may be achieved through the use of distinct PPAR-γ agonists.

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

Chemokines are small molecular weight (8–14 kDa) chemotactic cytokines that are produced locally at sites of inflammation and establish a concentration gradient resulting in the active recruitment of target cell populations [47]. Chemokines are a structurally and functionally related family of proteins subdivided into four groups based on the relative position of conserved N-terminal cysteine residues [47–49]. In general, the chemokine subfamilies show similar, often overlapping specificity with regards to the movements of the target cell populations they orchestrate. One key chemokine involved in the recruitment of neutrophils into areas of inflammation, including the CNS, is CXCL2 (MIP-2) [50–52]. The effects of 15d-PGJ_2 on microglial CXCL2 expression were complex and varied with each TLR ligand. Specifically, CXCL2 release was either enhanced (polyI:C, LPS, and ODN), reduced (Pam3Cys4), or remained unchanged (flagellin and ssRNA) (Figure 6). Increases in CXCL2 production were also observed following pioglitazone treatment in Pam3Cys- and ODN-stimulated microglia (Figure 7). The overall stimulatory activity of 15d-PGJ_2 on CXCL2 production is in agreement with reports from other groups [53, 54].

Another chemokine that is associated with mononuclear cell infiltration during various CNS infections is

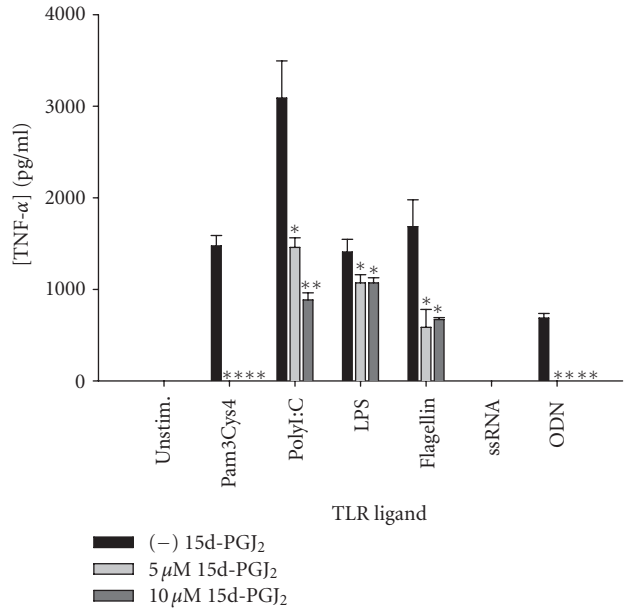

Figure 4: *The potency of 15d-PGJ$_2$ to attenuate TNF-α production varies according to the TLR ligand examined.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon TNF-α levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

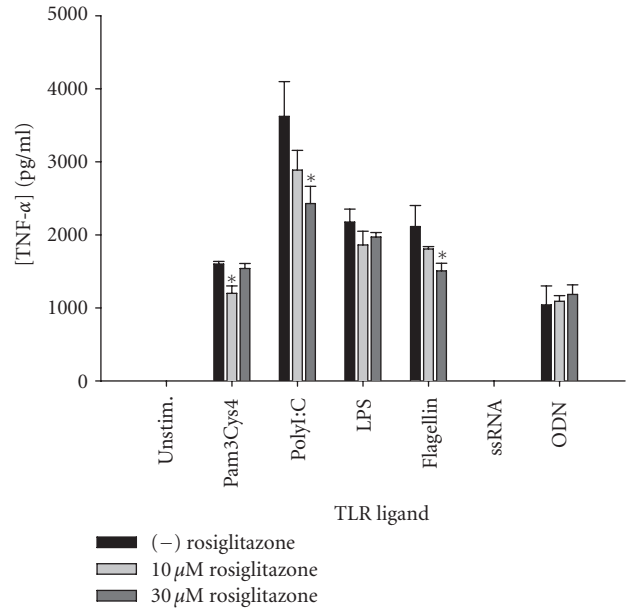

Figure 5: *The synthetic PPAR-γ agonist rosiglitazone selectively inhibits microglial TNF-α expression in response to TLR ligands.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of rosiglitazone prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon TNF-α levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + rosiglitazone are noted with asterisks ($^*P < .05$). Results presented are representative of two independent experiments.

CCL2 (MCP-1), which targets monocyte and lymphocyte entry [52, 55, 56]. Unlike CXCL2, which was differentially regulated by 15d-PGJ$_2$ in response to diverse TLR ligands, CCL2 production was uniformly and potently attenuated by this PPAR-γ agonist in response to the full array of TLR ligands tested (Figure 8). Similar to IL-12 p40 production, the synthetic TZDs demonstrated differential effects on CCL2 release from TLR ligand activated microglia. Specifically, rosiglitazone was fairly comprehensive in its ability to attenuate CCL2 production with significant reductions observed in response to Pam3Cys4, polyI:C, LPS, flagellin, and ODN, whereas the other TZDs tested (ciglitazone and pioglitazone) did not have much effect on CCL2 release in response to the majority of TLR ligands tested (data not shown). In summary, these results reveal that PPAR-γ agonists display a wide range of effects on chemokine production by microglia elicited by TLR ligands.

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

Astrocytes participate in CNS innate immune responses as evident by their ability to produce a wide array of inflammatory mediators in response to diverse stimuli [1, 4]. As already mentioned, these molecules can have dramatic consequences on CNS infection and tissue damage with net effects dictated by factors such as timing and duration of release. To determine the effects of PPAR-γ agonists on astrocyte responses to TLR ligands, we examined the production of two proinflammatory mediators produced by activated astrocytes, namely, NO and IL-12 p40. Both polyI:C and LPS were capable of reproducibly inducing NO expression in astrocytes as previously described [31, 57], which was attenuated by 15d-PGJ$_2$ in a dose-dependent manner (Figure 9). Similar inhibitory effects on astrocytic NO release in response to polyI:C and LPS were observed with troglitazone and ciglitazone, whereas rosiglitazone and pioglitazone did not modulate NO production (data not shown). Of note was the fact that unlike microglia, which exhibited significant cell death in response to the highest dose of 15d-PGJ$_2$ tested (i.e., 20 μM), astrocyte viability was not adversely affected by 15d-PGJ$_2$ at any of the concentrations examined. This finding is in agreement with previous reports demonstrating that, in general, primary astrocytes are more refractory to the toxic effects of PPAR-γ agonists compared to primary microglia [23, 24, 27].

Similar to the findings obtained with microglia, 15d-PGJ$_2$ was a universal and potent inhibitor of IL-12 p40 production by astrocytes in response to all TLR ligands tested

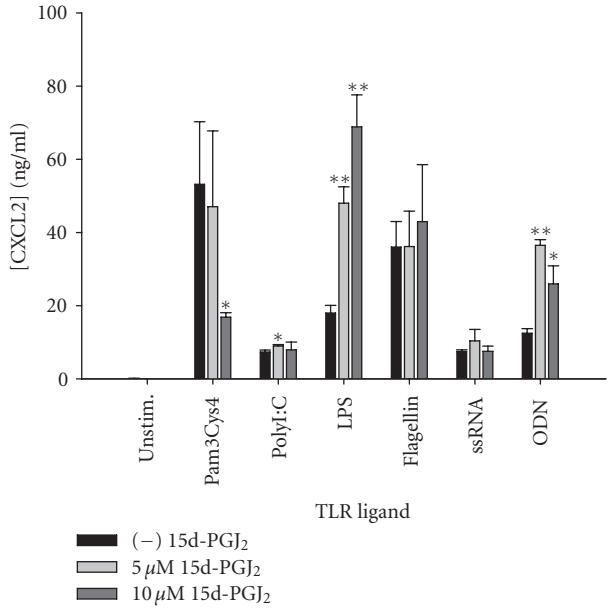

Figure 6: *15d-PGJ$_2$ demonstrates differential effects on CXCL2 production by microglia, which are TLR ligand-dependent.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CXCL2 levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

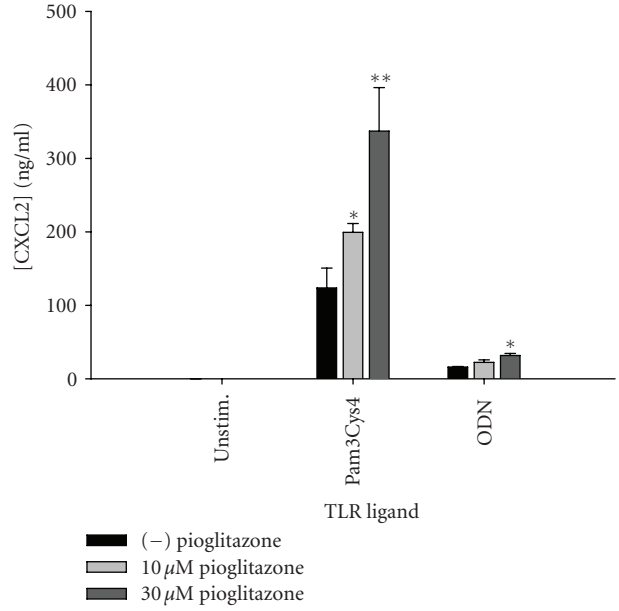

Figure 7: *Pioglitazone augments microglial CXCL2 production in response to Pam3Cys4 and ODN.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone prior to the addition of Pam3Cys4 or ODN. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CXCL2 levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + pioglitazone are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

(Figure 10). In addition, both pioglitazone and troglitazone were capable of attenuating IL-12 p40 expression in response to the full array of TLR ligands examined (Figure 11 and data not shown), whereas the effects of ciglitazone were variable.

3.4. PPAR-γ agonists modulate chemokine production by astrocytes following TLR ligand exposure

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

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

4. DISCUSSION

In order for the CNS to respond to infectious insults, a rapid and efficient host immune response must be initiated and directed to expedite pathogen elimination. One means to achieve this goal is through the triggering of TLRs expressed on resident glia that signal proinflammatory mediator release in an attempt to quell infection [11, 12]. However, recent evidence also suggests that these normally protective immune responses can become dysregulated, culminating

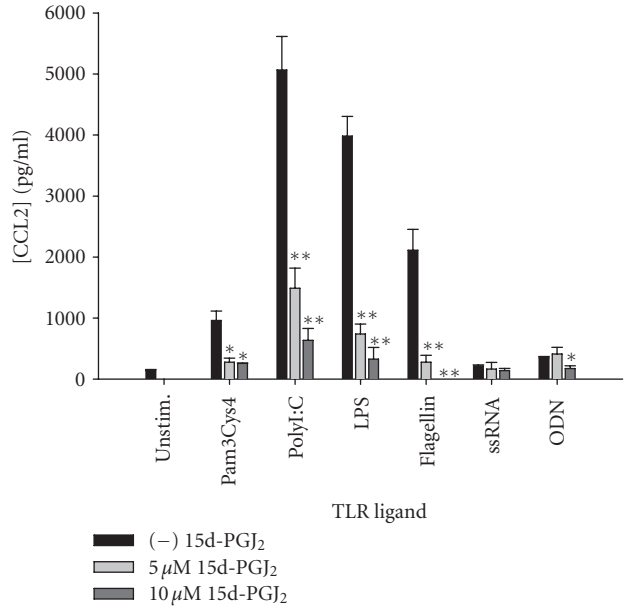

Figure 8: *15d-PGJ$_2$ is a potent inhibitor of CCL2 release by microglia in response to a wide range of TLR ligands.* Primary microglia were plated at 2×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CCL2 levels were determined by ELISA. Significant differences between microglia treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

in the destruction of surrounding normal CNS parenchyma [13]. Therefore, fine tuning the resultant immune response to achieve maximal pathogen clearance concomitant with minimal tissue damage would represent a best case scenario for the management of a wide array of CNS infectious diseases including bacterial meningitis, HIVE, brain abscess, and other viral infections. The purpose of this study was to perform a comprehensive analysis of the ability of several PPAR-γ agonists to regulate glial activation in response to a panel of TLR ligands that may be encountered during native CNS infections. It is envisioned that this information may be exploited as a first step towards the derivation of specific treatment strategies that could be implemented with existing therapies for CNS infections to maximize benefit to patients.

One obvious distinction between the various PPAR-γ agonists tested to modulate TLR-dependent glial activation was the finding that 15d-PGJ$_2$ consistently led to more dramatic and widespread decreases in inflammatory mediator production compared to the synthetic TZDs. This relationship was observed with both primary microglia and astrocytes and is in agreement with previous reports by us and other laboratories where 15d-PGJ$_2$ exerted potent inhibitory effects at lower effective concentrations compared to TZDs, despite the fact that the former exhibits a lower binding affinity to PPAR-γ [26, 27, 58, 59]. In addition, differences were observed between the immune modulatory effects within the group of TZDs tested. This was somewhat surprising; however, Storer et al. also reported that ciglitazone and pioglitazone had no effect on TNF-α or CCL2 production by microglia in response to LPS [27], similar to our findings in the present study. However, a few discrepancies between these reports also exist, which might be explained by the fact that only a single TLR ligand was examined (i.e., LPS) and the concentrations of several TZDs exceeded the maximal dose tested in the current study. In general, only one TZD, rosiglitazone, exerted rather global effects on the inflammatory mediators examined here, whereas the other compounds (i.e., ciglitazone, troglitazone, and pioglitazone) demonstrated differential effects that were dependent on the TLR ligand as well as the proinflammatory mediator measured. The reason responsible for these differences is not clear but distinct chemical structures of the various TZDs have been suggested to contribute to their unique properties [60]. The widespread effects of rosiglitazone on glial inflammatory mediator production are in agreement with the fact that this TZD has been reported to impact numerous PPAR-γ isoforms including PPAR-γ1 and PPAR-γ2, whereas other TZDs have been reported to selectively target a single PPAR-γ subtype [61, 62]. Other studies have revealed distinct differences in the effectiveness of TZD members in regulating changes in glucose metabolism in astrocytes [63] and mitochondrial function [64]. In addition, the EC_{50} of individual TZD compounds for PPAR-γ does vary within this group of molecules depending on the experimental readout examined, which may contribute to their differential effects [61, 62]. Alternatively, studies have shown that the coactivator proteins interacting with PPAR-γ differ in a ligand-dependent manner [65]. Unfortunately, there are few reports where comprehensive side-by-side comparisons have been made with regard to the effects of various TZD compounds on neuroinflammation either in vitro or in vivo. Making direct comparisons between various TZDs in disparate models of neuroinflammation should be viewed cautiously since differences in disease models, inflammatory stimuli, and species examined all have the potential to influence the results obtained.

Our findings demonstrating the inhibitory effects of 15d-PGJ$_2$ on TLR ligand-induced glial activation are similar to those observed following LPS stimulation. Specifically, 15d-PGJ$_2$ has been shown to inhibit LPS-induced NO [27–29, 66], TNF-α [27, 28, 66], and IL-12 family member expression [26] in microglia and/or astrocytes. Importantly, this report has provided a more comprehensive analysis of the effects of PPAR-γ agonists on glial activation with the inclusion of a battery of TLR ligands that would be encountered during various CNS infectious diseases. Earlier studies have primarily focused on the immune modulatory effects of PPAR-γ agonists in response to the TLR4 ligand LPS, and therefore, this study is also novel from the perspective that numerous TLR ligands were evaluated.

Interestingly, both 15d-PGJ$_2$ and pioglitazone were found to augment CXCL2 release by microglia and astrocytes in response to specific TLR ligands. In particular, 15d-PGJ$_2$ enhanced CXCL2 production in response to LPS stimulation

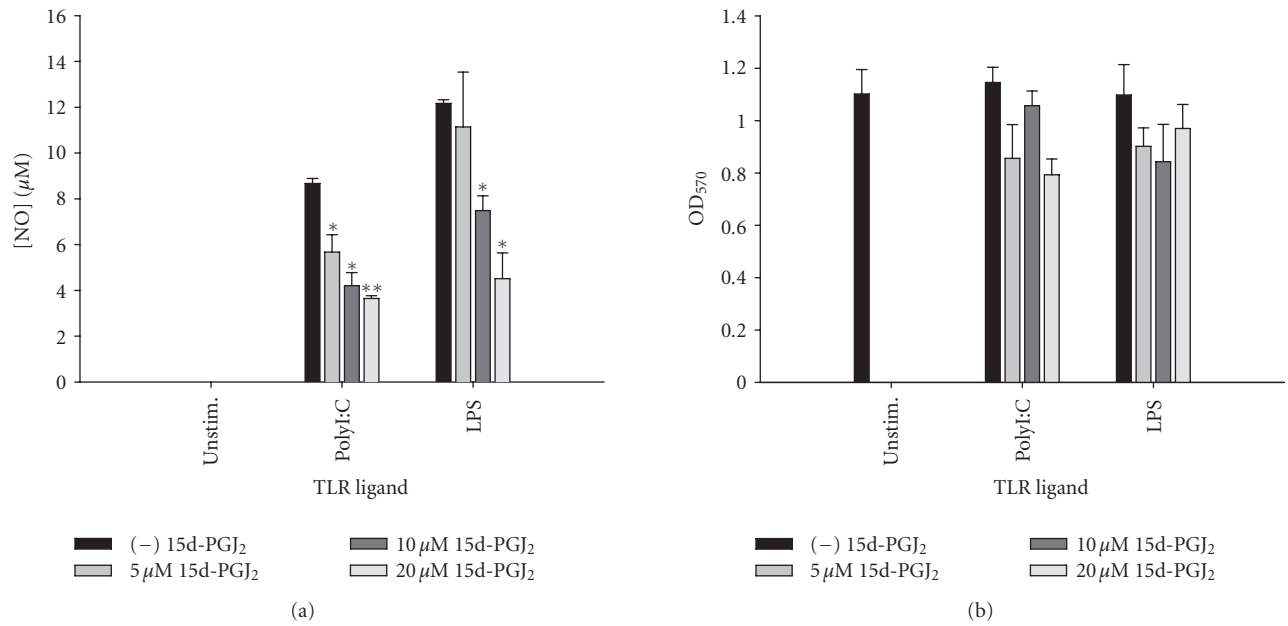

Figure 9: *15d-PGJ$_2$ attenuates NO production by astrocytes in response to polyI:C and LPS stimulation.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of polyI:C or LPS. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon NO levels were determined by the Griess reagent (a). The effects of 15d-PGJ$_2$ on astrocyte viability were assessed using an MTT assay (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

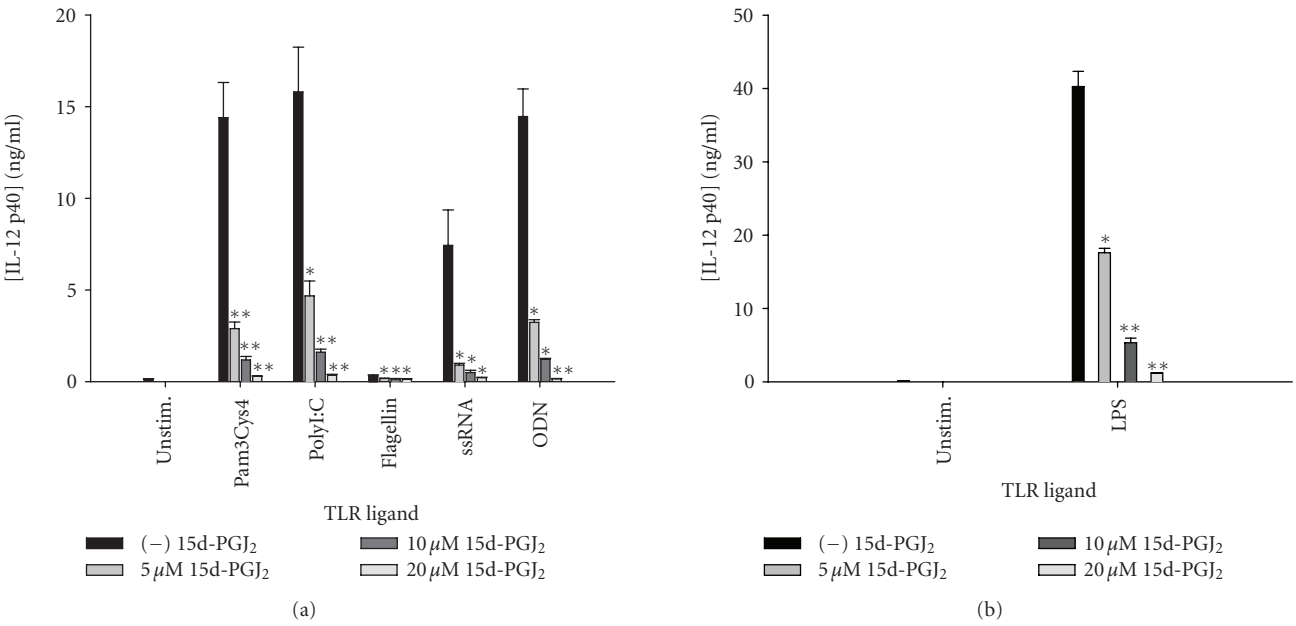

Figure 10: *15d-PGJ$_2$ is a global inhibitor of astrocytic IL-12 p40 release following TLR ligand exposure.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$ prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon IL-12 p40 levels were determined by ELISA (a) and (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + 15d-PGJ$_2$ are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

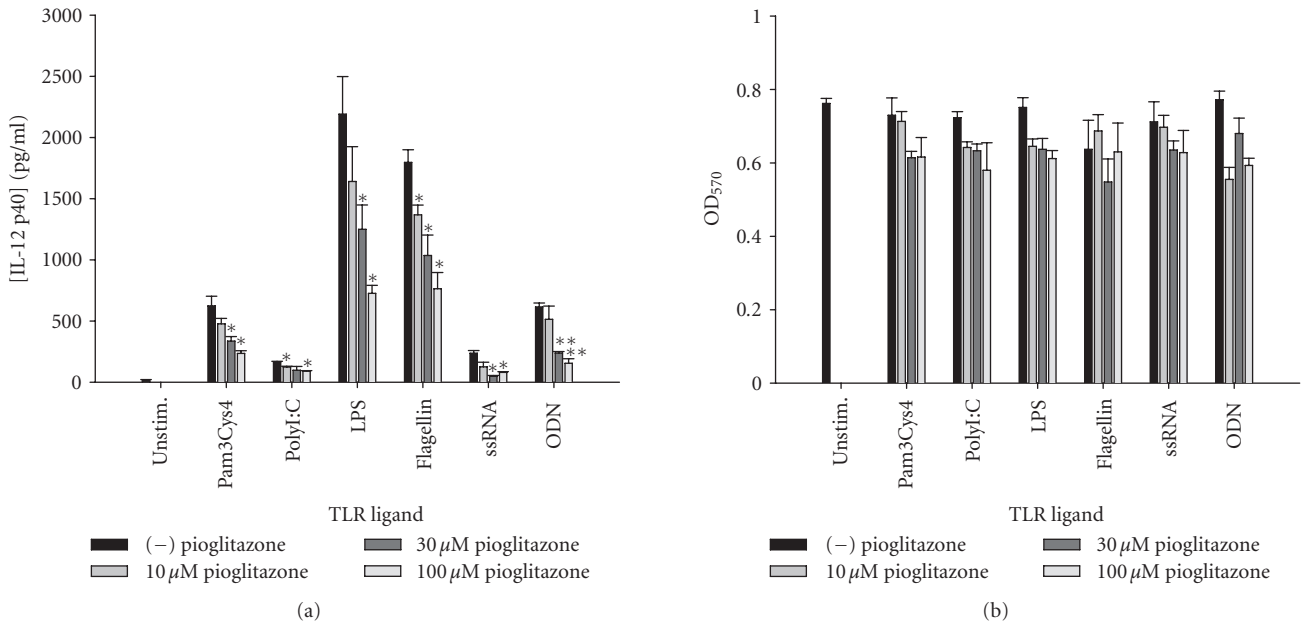

Figure 11: *The TZD pioglitazone inhibits astrocytic IL-12 p40 expression in response to diverse TLR ligands.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone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

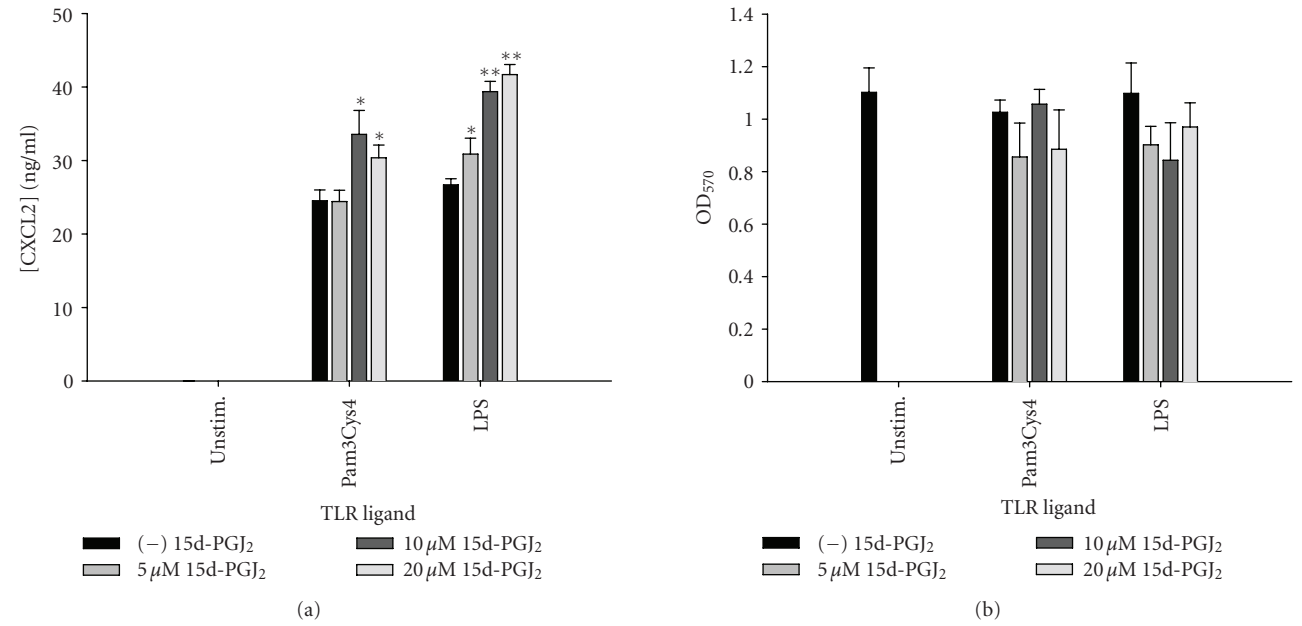

Figure 12: *CXCL2 release is augmented in astrocytes by 15d-PGJ$_2$ in response to distinct TLR ligands.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of 15d-PGJ$_2$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

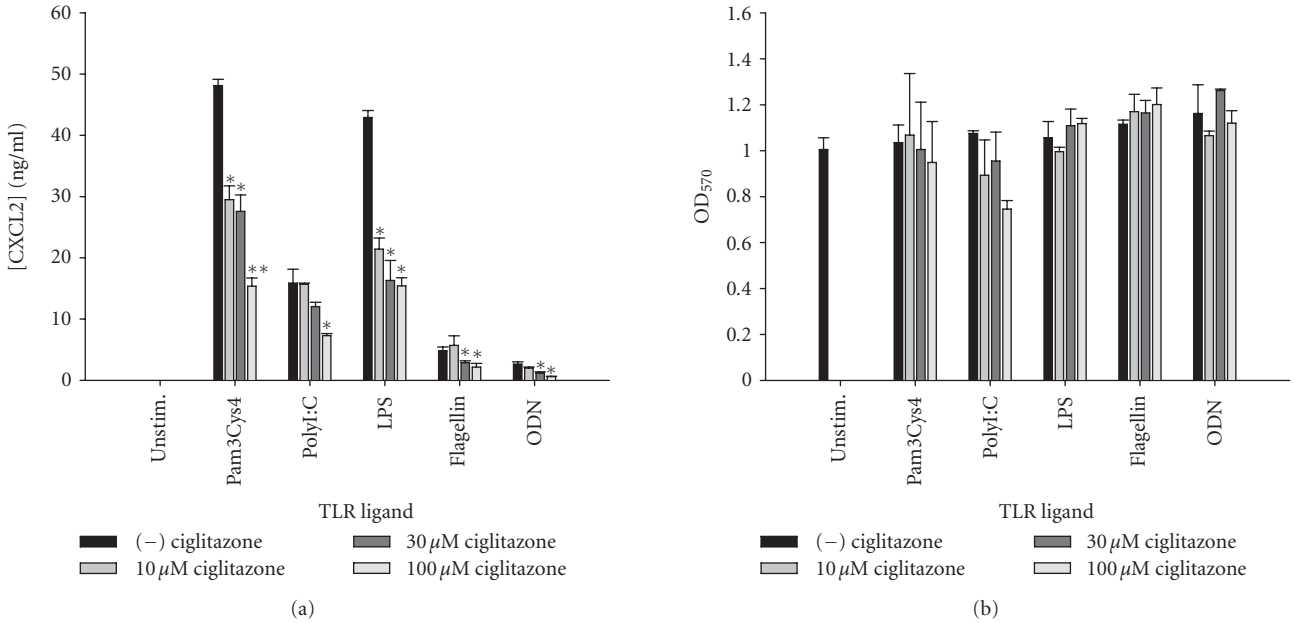

Figure 13: *Ciglitazone attenuates astrocytic CXCL2 expression in response to several TLR ligands.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of ciglitazone prior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

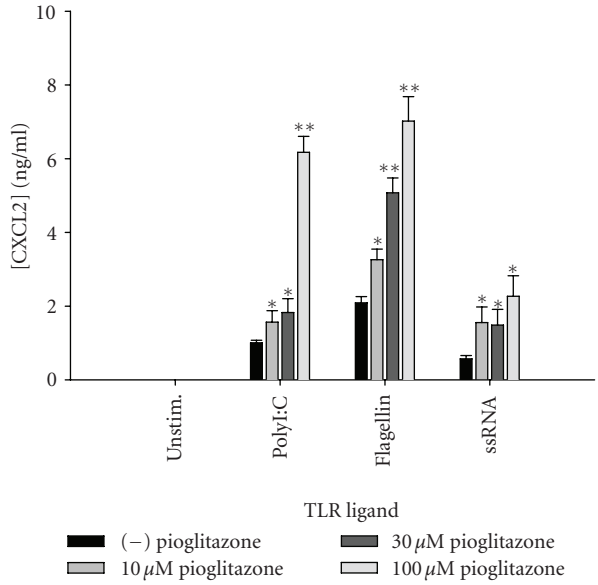

Figure 14: *Pioglitazone enhances CXCL2 release by astrocytes following TLR ligand exposure.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone prior to the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

in both microglia and astrocytes, in agreement with previous reports [53, 54]. In addition, recent results from our laboratory have revealed that CXCL2 release was slightly increased by 15d-PGJ_2 and downregulated by ciglitazone in response to the gram-positive pathogen *S. aureus* in primary astrocytes [24], in corroboration with the current report.

Elevated levels of endogenous 15d-PGJ_2 have been associated with the resolution of inflammation in vivo, suggesting that it functions as negative feedback regulator of inflammatory responses [67, 68]. This study demonstrates that 15d-PGJ_2 is an effective and selective inhibitor of glial activation in response to TLR ligands, suggesting that it may be capable of modulating chronic microglial and astrocyte responses during the course of CNS infectious diseases. Evidence to support this concept is provided by our findings that 15d-PGJ_2 effectively inhibited IL-12 p40 and CCL2 expression in microglia and astrocytes, two molecules with important functions in the differentiation of $CD4^+$ Th1 cells and monocyte and T cell influx into the infected CNS [52, 55, 56, 69, 70]. It is possible that the downregulation of these mediators in microglia and astrocytes following 15d-PGJ_2 treatment results in the failure to recruit and/or stimulate Ag-specific T cells in the CNS parenchyma. Therefore, preventing chronic microglial activation by 15d-PGJ_2 or synthetic PPAR-γ agonists may help to resolve inflammation earlier, resulting in less damage to surrounding normal brain parenchyma.

In summary, these studies demonstrate that not all PPAR-γ agonists are created equal in terms of their ability to modulate proinflammatory mediator release by activated microglia and astrocytes. Specifically, differences were

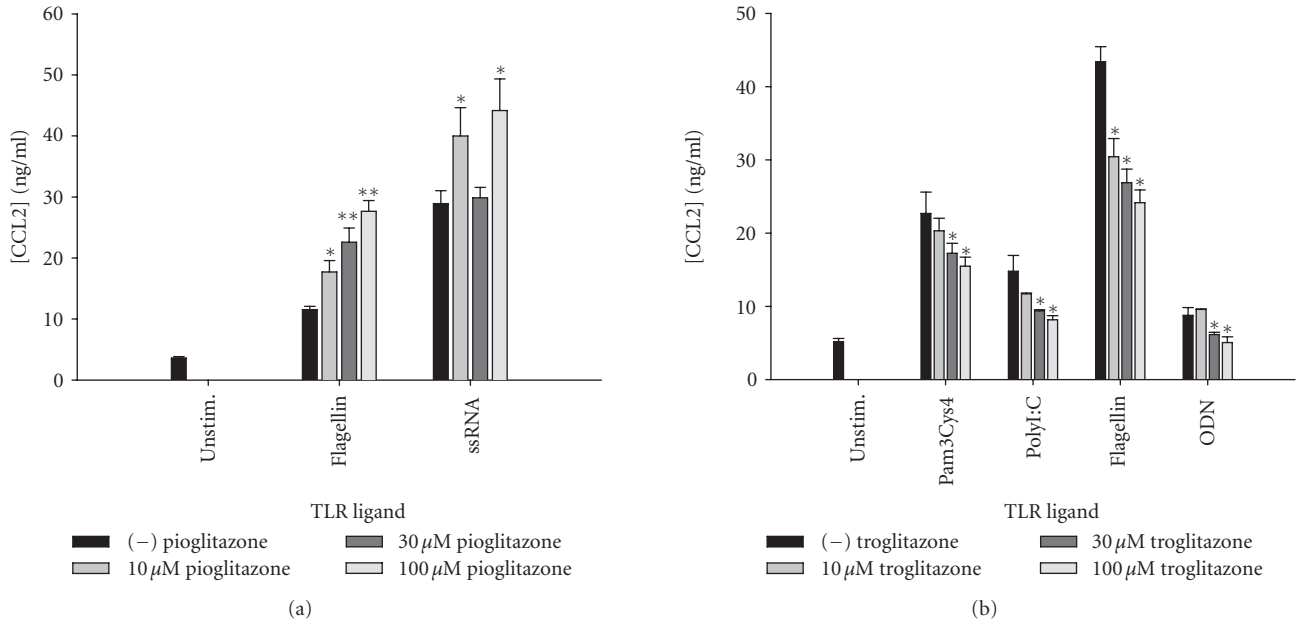

Figure 15: *TZDs exert differential effects on astrocytic CCL2 production following TLR ligand treatment.* Primary astrocytes were plated at 1×10^5 cells/well in 96-well plates and incubated overnight. The following day, cells were pretreated for 1 hour with the indicated concentrations of pioglitazone (a) or troglitazone, (b) prior to the addition of TLR ligands. Cell-conditioned supernatants were collected at 24 hours following TLR ligand exposure, whereupon CCL2 levels were determined by ELISA (a) and (b). Significant differences between astrocytes treated with TLR ligands alone versus TLR ligands + TZDs are noted with asterisks ($^*P < .05$; $^{**}P < .001$). Results presented are representative of two independent experiments.

specific to the type of TLR ligand examined. When considering the potential utility of PPAR-γ agonists for modulating pathological inflammation typical of several CNS infectious diseases, critical issues such as the timing and length of PPAR-γ administration and doses of compound must be considered. For example, it appears likely that compounds should be delivered at periods at or nearing pathogen clearance since a significant attenuation of the host immune response would be counterproductive to infection resolution. Upon pathogen elimination, PPAR-γ agonists may minimize damage to surrounding tissue by downregulating exaggerated CNS immune responses that could be perpetuated by microbial debris (i.e., cell wall fragments such as LPS and PGN or pathogen nucleic acids) via continued engagement of TLRs. Indeed, recent studies by our laboratory have revealed that the PPAR-γ agonist ciglitazone demonstrates beneficial effects with delayed administration in an experimental brain abscess model as revealed by accelerated abscess encapsulation and reduction in bacterial burdens [71]. In addition, recent studies by other groups have revealed beneficial effects of PPAR-γ agonists in other infectious disease paradigms [72–76]. Therefore, the current study can be used as a guide to facilitate the selection of PPAR-γ agonists as candidates for intervention during CNS infectious diseases.

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REFERENCES

[1] C. Farina, F. Aloisi, and E. Meinl, "Astrocytes are active players in cerebral innate immunity," *Trends in Immunology*, vol. 28, no. 3, pp. 138–145, 2007.

[2] F. Aloisi, "Immune function of microglia," *Glia*, vol. 36, no. 2, pp. 165–179, 2001.

[3] U.-K. Hanisch, "Microglia as a source and target of cytokines," *Glia*, vol. 40, no. 2, pp. 140–155, 2002.

[4] Y. Dong and E. N. Benveniste, "Immune function of astrocytes," *Glia*, vol. 36, no. 2, pp. 180–190, 2001.

[5] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[6] T. Kaisho and S. Akira, "Pleiotropic function of Toll-like receptors," *Microbes and Infection*, vol. 6, no. 15, pp. 1388–1394, 2004.

[7] S. Carpenter and L. A. J. O'Neill, "How important are Toll-like receptors for antimicrobial responses?" *Cellular Microbiology*, vol. 9, no. 8, pp. 1891–1901, 2007.

[8] G. Sitia, M. Iannacone, S. Müller, M. E. Bianchi, and L. G. Guidotti, "Treatment with HMGB1 inhibitors diminishes CTL-induced liver disease in HBV transgenic mice," *Journal of Leukocyte Biology*, vol. 81, no. 1, pp. 100–107, 2007.

[9] A. M. Krieg and J. Vollmer, "Toll-like receptors 7, 8, and 9: linking innate immunity to autoimmunity," *Immunological Reviews*, vol. 220, no. 1, pp. 251–269, 2007.

[10] B. J. Marsland and M. Kopf, "Toll-like receptors: paving the path to T cell-driven autoimmunity?" *Current Opinion in Immunology*, vol. 19, no. 6, pp. 611–614, 2007.

[11] T. Kielian, "Toll-like receptors in central nervous system glial inflammation and homeostasis," *Journal of Neuroscience Research*, vol. 83, no. 5, pp. 711–730, 2006.

[12] P. A. Carpentier, D. S. Duncan, and S. D. Miller, "Glial toll-like receptor signaling in central nervous system infection and autoimmunity," *Brain, Behavior, and Immunity*, vol. 22, no. 2, pp. 140–147, 2008.

[13] P. H. Larsen, T. H. Holm, and T. Owens, "Toll-like receptors in brain development and homeostasis," *Science's STKE*, vol. 2007, no. 402, p. pe47, 2007.

[14] A. A. Babcock, M. Wirenfeldt, T. Holm, et al., "Toll-like receptor 2 signaling in response to brain injury: an innate bridge to neuroinflammation," *Journal of Neuroscience*, vol. 26, no. 49, pp. 12826–12837, 2006.

[15] K. A. Kigerl, W. Lai, S. Rivest, R. P. Hart, A. R. Satoskar, and P. G. Popovich, "Toll-like receptor (TLR)-2 and TLR-4 regulate inflammation, gliosis, and myelin sparing after spinal cord injury," *Journal of Neurochemistry*, vol. 102, no. 1, pp. 37–50, 2007.

[16] T. Kielian and P. D. Drew, "Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation," *Journal of Neuroscience Research*, vol. 71, no. 3, pp. 315–325, 2003.

[17] J. N. Feige, L. Gelman, L. Michalik, B. Desvergne, and W. Wahli, "From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions," *Progress in Lipid Research*, vol. 45, no. 2, pp. 120–159, 2006.

[18] R. Kapadia, J.-H. Yi, and R. Vemuganti, "Mechanisms of anti-inflammatory and neuroprotective actions of PPAR-gamma agonists," *Frontiers in Bioscience*, vol. 13, no. 5, pp. 1813–1826, 2008.

[19] R. Kostadinova, W. Wahli, and L. Michalik, "PPARs in diseases: control mechanisms of inflammation," *Current Medicinal Chemistry*, vol. 12, no. 25, pp. 2995–3009, 2005.

[20] M. T. Heneka and G. E. Landreth, "PPARs in the brain," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 1031–1045, 2007.

[21] P. D. Drew, J. Xu, P. D. Storer, J. A. Chavis, and M. K. Racke, "Peroxisome proliferator-activated receptor agonist regulation of glial activation: relevance to CNS inflammatory disorders," *Neurochemistry International*, vol. 49, no. 2, pp. 183–189, 2006.

[22] A. Bernando and L. Minghetti, "PPAR-γ agonists as regulators of microglial activation and brain inflammation," *Current Pharmaceutical Design*, vol. 12, no. 1, pp. 93–109, 2006.

[23] T. Kielian, M. McMahon, E. D. Bearden, A. C. Baldwin, P. D. Drew, and N. Esen, "*S. aureus*-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2)," *Journal of Neurochemistry*, vol. 90, no. 5, pp. 1163–1172, 2004.

[24] N. K. Phulwani, D. L. Feinstein, V. Gavrilyuk, C. Akar, and T. Kielian, "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," *Journal of Neurochemistry*, vol. 99, no. 5, pp. 1389–1402, 2006.

[25] Q. Jiang, M. Heneka, and G. E. Landreth, "The role of peroxisome proliferator-activated receptor-γ (PPARγ) in Alzheimer's disease: therapeutic implications," *CNS Drugs*, vol. 22, no. 1, pp. 1–14, 2008.

[26] J. Xu and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia," *Journal of Immunology*, vol. 178, no. 3, pp. 1904–1913, 2007.

[27] P. D. Storer, J. Xu, J. Chavis, and P. D. Drew, "Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis," *Journal of Neuroimmunology*, vol. 161, no. 1-2, pp. 113–122, 2005.

[28] A. Bernardo, G. Levi, and L. Minghetti, "Role of the peroxisome proliferator-activated receptor-γ (PPAR-γ) and its natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in the regulation of microglial functions," *European Journal of Neuroscience*, vol. 12, no. 7, pp. 2215–2223, 2000.

[29] T. V. Petrova, K. T. Akama, and L. J. Van Eldik, "Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 8, pp. 4668–4673, 1999.

[30] C. S. Jack, N. Arbour, J. Manusow, et al., "TLR signaling tailors innate immune responses in human microglia and astrocytes," *Journal of Immunology*, vol. 175, no. 7, pp. 4320–4330, 2005.

[31] P. A. Carpentier, W. S. Begolka, J. K. Olson, A. Elhofy, W. J. Karpus, and S. D. Miller, "Differential activation of astrocytes by innate and adaptive immune stimuli," *Glia*, vol. 49, no. 3, pp. 360–374, 2005.

[32] J. K. Olson and S. D. Miller, "Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs," *Journal of Immunology*, vol. 173, no. 6, pp. 3916–3924, 2004.

[33] N. Esen, F. Y. Tanga, J. A. DeLeo, and T. Kielian, "Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the Gram-positive bacterium *Staphylococcus aureus*," *Journal of Neurochemistry*, vol. 88, no. 3, pp. 746–758, 2004.

[34] N. Esen and T. Kielian, "Central role for MyD88 in the responses of microglia to pathogen-associated molecular patterns," *Journal of Immunology*, vol. 176, no. 11, pp. 6802–6811, 2006.

[35] N. Esen and T. Kielian, "Effects of low dose GM-CSF on microglial inflammatory profiles to diverse pathogen-associated molecular patterns (PAMPs)," *Journal of Neuroinflammation*, vol. 4, article 10, 2007.

[36] N. Esen, D. Shuffield, M. M. Syed, and T. Kielian, "Modulation of connexin expression and gap junction communication in astrocytes by the gram-positive bacterium *S. aureus*," *Glia*, vol. 55, no. 1, pp. 104–117, 2007.

[37] M. Bsibsi, R. Ravid, D. Gveric, and J. M. van Noort, "Broad expression of Toll-like receptors in the human central nervous system," *Journal of Neuropathology & Experimental Neurology*, vol. 61, no. 11, pp. 1013–1021, 2002.

[38] T. Kielian, N. Esen, and E. D. Bearden, "Toll-like receptor 2 (TLR2) is pivotal for recognition of *S. aureus* peptidoglycan but not intact bacteria by microglia," *Glia*, vol. 49, no. 4, pp. 567–576, 2005.

[39] S. C. Byram, M. J. Carson, C. A. DeBoy, C. J. Serpe, V. M. Sanders, and K. J. Jones, "CD4-positive T cell-mediated neuroprotection requires dual compartment antigen presentation," *Journal of Neuroscience*, vol. 24, no. 18, pp. 4333–4339, 2004.

[40] M. J. Carson, T. V. Bilousova, S. S. Puntambekar, B. Melchior, J. M. Doose, and I. M. Ethell, "A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease," *Neurotherapeutics*, vol. 4, no. 4, pp. 571–579, 2007.

[41] N. P. Turrin and S. Rivest, "Molecular and cellular immune mediators of neuroprotection," *Molecular Neurobiology*, vol. 34, no. 3, pp. 221–242, 2006.

[42] D. Gosselin and S. Rivest, "Role of IL-1 and TNF in the brain: twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system," *Brain, Behavior, and Immunity*, vol. 21, no. 3, pp. 281–289, 2007.

[43] K. Sriram and J. P. O'Callaghan, "Divergent roles for tumor necrosis factor-α in the brain," *Journal of NeuroImmune Pharmacology*, vol. 2, no. 2, pp. 140–153, 2007.

[44] T. Kielian, "Immunopathogenesis of brain abscess," *Journal of Neuroinflammation*, vol. 1, article 16, 2004.

[45] R. Nau and W. Brück, "Neuronal injury in bacterial meningitis: mechanisms and implications for therapy," *Trends in Neurosciences*, vol. 25, no. 1, pp. 38–45, 2002.

[46] R. N. Saha and K. Pahan, "Tumor necrosis factor-α at the crossroads of neuronal life and death during HIV-associated dementia," *Journal of Neurochemistry*, vol. 86, no. 5, pp. 1057–1071, 2003.

[47] R. M. Ransohoff, L. Liu, and A. E. Cardona, "Chemokines and chemokine receptors: multipurpose players in neuroinflammation," *International Review of Neurobiology*, vol. 82, pp. 187–204, 2007.

[48] A. D. Luster, "Chemokines—chemotactic cytokines that mediate inflammation," *The New England Journal of Medicine*, vol. 338, no. 7, pp. 436–445, 1998.

[49] N. M. Rebenko-Moll, L. Liu, A. Cardona, and R. M. Ransohoff, "Chemokines, mononuclear cells and the nervous system: heaven (or hell) is in the details," *Current Opinion in Immunology*, vol. 18, no. 6, pp. 683–689, 2006.

[50] T. Kielian, B. Barry, and W. F. Hickey, "CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses," *Journal of Immunology*, vol. 166, no. 7, pp. 4634–4643, 2001.

[51] M. D. Bell, D. D. Taub, S. J. Kunkel, et al., "Recombinant human adenovirus with rat *MIP-2* gene insertion causes prolonged PMN recruitment to the murine brain," *European Journal of Neuroscience*, vol. 8, no. 9, pp. 1803–1811, 1996.

[52] R. M. Ransohoff, P. Kivisäkk, and G. Kidd, "Three or more routes for leukocyte migration into the central nervous system," *Nature Reviews Immunology*, vol. 3, no. 7, pp. 569–581, 2003.

[53] X. Zhang, J. M. Wang, W. H. Gong, N. Mukaida, and H. A. Young, "Differential regulation of chemokine gene expression by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2," *Journal of Immunology*, vol. 166, no. 12, pp. 7104–7111, 2001.

[54] Q. Si, M.-L. Zhao, A. C. Morgan, C. F. Brosnan, and S. C. Lee, "15-deoxy-$\Delta^{12,14}$,-prostaglandin J_2 inhibits IFN-inducible protein 10/CXC chemokine ligand 10 expression in human microglia: mechanisms and implications," *Journal of Immunology*, vol. 173, no. 5, pp. 3504–3513, 2004.

[55] T. Kielian, "Microglia and chemokines in infectious diseases of the nervous system: views and reviews," *Frontiers in Bioscience*, vol. 9, pp. 732–750, 2004.

[56] D. J. Mahad and R. M. Ransohoff, "The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE)," *Seminars in Immunology*, vol. 15, no. 1, pp. 23–32, 2003.

[57] C. J. Auch, R. N. Saha, F. G. Sheikh, X. Liu, B. L. Jacobs, and K. Pahan, "Role of protein kinase R in double-stranded RNA-induced expression of nitric oxide synthase in human astroglia," *FEBS Letters*, vol. 563, no. 1–3, pp. 223–228, 2004.

[58] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[59] D. S. Straus and C. K. Glass, "Cyclopentenone prostaglandins: new insights on biological activities and cellular targets," *Medicinal Research Reviews*, vol. 21, no. 3, pp. 185–210, 2001.

[60] L. Van Gaal and A. J. Scheen, "Are all glitazones the same?" *Diabetes/Metabolism Research and Reviews*, vol. 18, supplement 2, pp. S1–S4, 2002.

[61] T. M. Willson, J. E. Cobb, D. J. Cowan, et al., "The structure-activity relationship between peroxisome proliferator-activated receptor γ agonism and the antihyperglycemic activity of thiazolidinediones," *Journal of Medicinal Chemistry*, vol. 39, no. 3, pp. 665–668, 1996.

[62] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[63] C. Dello Russo, V. Gavrilyuk, G. Weinberg, et al., "Peroxisome proliferator-activated receptor γ thiazolidinedione agonists increase glucose metabolism in astrocytes," *Journal of Biological Chemistry*, vol. 278, no. 8, pp. 5828–5836, 2003.

[64] D. L. Feinstein, A. Spagnolo, C. Akar, et al., "Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key?" *Biochemical Pharmacology*, vol. 70, no. 2, pp. 177–188, 2005.

[65] Y. Kodera, K. Takeyama, A. Murayama, M. Suzawa, Y. Masuhiro, and S. Kato, "Ligand type-specific interactions of peroxisome proliferator-activated receptor γ with transcriptional coactivators," *Journal of Biological Chemistry*, vol. 275, no. 43, pp. 33201–33204, 2000.

[66] P. D. Drew and J. A. Chavis, "The cyclopentone prostaglandin 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 represses nitric oxide, TNF-α, and IL-12 production by microglial cells," *Journal of Neuroimmunology*, vol. 115, no. 1-2, pp. 28–35, 2001.

[67] D. W. Gilroy, P. R. Colville-Nash, D. Willis, J. Chivers, M. J. Paul-Clark, and D. A. Willoughby, "Inducible cyclooxygenase may have anti-inflammatory properties," *Nature Medicine*, vol. 5, no. 6, pp. 698–701, 1999.

[68] T. Lawrence, D. A. Willoughby, and D. W. Gilroy, "Anti-inflammatory lipid mediators and insights into the resolution of inflammation," *Nature Reviews Immunology*, vol. 2, no. 10, pp. 787–795, 2002.

[69] C. L. Langrish, B. S. McKenzie, N. J. Wilson, R. de Waal Malefyt, R. A. Kastelein, and D. J. Cua, "IL-12 and IL-23: master regulators of innate and adaptive immunity," *Immunological Reviews*, vol. 202, no. 1, pp. 96–105, 2004.

[70] G. Trinchieri, "Interleukin-12 and the regulation of innate resistance and adaptive immunity," *Nature Reviews Immunology*, vol. 3, no. 2, pp. 133–146, 2003.

[71] T. Kielian, M. M. Syed, S. Liu, N. Phillips, G. Wagoner, P. D. Drew, and N. Esen, "The synthetic PPAR-γ agonist ciglitazone attenuates neuroinflammation and accelerates encapsulation in bacterial brain abscesses," *Journal of immunology*, vol. 180, no. 3, pp. 5004–5016, 2008.

[72] A. von Knethen, M. Soller, and B. Brüne, "Peroxisome proliferator-activated receptor γ (PPARγ) and sepsis," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 55, no. 1, pp. 19–25, 2007.

[73] R. Arnold and W. König, "Peroxisome proliferator-activated receptor-γ agonists inhibit the replication of respiratory

syncytial virus (RSV) in human lung epithelial cells," *Virology*, vol. 350, no. 2, pp. 335–346, 2006.

[74] D. Liu, B. X. Zeng, S. H. Zhang, and S. L. Yao, "Rosiglitazone, an agonist of peroxisome proliferator-activated receptor γ, reduces pulmonary inflammatory response in a rat model of endotoxemia," *Inflammation Research*, vol. 54, no. 11, pp. 464–470, 2005.

[75] J. M. Kaplan, J. A. Cook, P. W. Hake, M. O'Connor, T. J. Burroughs, and B. Zingarelli, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15D-PGJ_2), a peroxisome proliferator activated receptor γ ligand, reduces tissue leukosequestration and mortality in endotoxic shock," *Shock*, vol. 24, no. 1, pp. 59–65, 2005.

[76] S. H. Ramirez, D. Heilman, B. Morsey, R. Potula, J. Haorah, and Y. Persidsky, "Activation of peroxisome proliferator-activated receptor γ (PPARγ) suppresses Rho GTPases in human brain microvascular endothelial cells and inhibits adhesion and transendothelial migration of HIV-1 infected monocytes," *Journal of immunology*, vol. 180, no. 3, pp. 1854–1865, 2008.

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Research Article

The PPAR-γ Agonist 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 Attenuates Microglial Production of IL-12 Family Cytokines: Potential Relevance to Alzheimer's Disease

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Accumulation of amyloid-β peptide (Aβ) appears to contribute to the pathogenesis of Alzheimer's disease (AD). Therapeutic hope for the prevention or removal of Aβ deposits has been placed in strategies involving immunization against the Aβ peptide. Initial Aβ immunization studies in animal models of AD showed great promise. However, when this strategy was attempted in human subjects with AD, an unacceptable degree of meningoencephalitis occurred. It is generally believed that this adverse outcome resulted from a T-cell response to Aβ. Specifically, $CD4^+$ Th1 and Th17 cells may contribute to severe CNS inflammation and limit the utility of Aβ immunization in the treatment of AD. Interleukin (IL)-12 and IL-23 play critical roles in the development of Th1 and Th17 cells, respectively. In the present study, $A\beta_{1\text{-}42}$ synergistically elevated the expression of IL-12 and IL-23 triggered by inflammatory activation of microglia, and the peroxisome proliferator-activated receptor (PPAR)-γ agonist 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) effectively blocked the elevation of these proinflammatory cytokines. Furthermore, 15d-PGJ_2 suppressed the Aβ-related synergistic induction of CD14, MyD88, and Toll-like receptor 2, molecules that play critical roles in neuroinflammatory conditions. Collectively, these studies suggest that PPAR-γ agonists may be effective in modulating the development of AD.

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common cause of dementia in the elderly. AD is characterized by progressive memory deficits, changes in personality, and cognitive decline. It is believed that abnormal accumulation of amyloid-β peptide (Aβ), either as a soluble factor or as extracellular aggregates, contributes to the development of AD [1–3]. Cleavage of amyloid precursor protein (APP) can produce amyloid-β peptide 1-42 ($A\beta_{1\text{-}42}$), the levels of which are correlated with neurotoxicity and development of AD. The connection between Aβ and AD symptoms is further strengthened by mouse models in which transgenic expression of the human Aβ precursor (APP) results in accumulation of Aβ and deficits in memory tests [4]. Preclinical investigations of anti-Aβ therapies have come to rely on such mice as a loose approximation of AD pathogenesis. The most successful anti-Aβ strategy demonstrated in these mice to date involves recruiting the immune system through vaccination. APP-transgenic mice that are immunized against Aβ at a young age never develop substantial Aβ deposits, and vaccination after deposition can even reverse a significant degree of the Aβ accumulation [5]. Most importantly, behavioral deficits are alleviated by such immunizations. These benefits correlate strongly with the titers of soluble antibody generated against Aβ [6–8], and passive immunization by injection of anti-Aβ antibody alone is also effective [9, 10]. Unfortunately, the first attempt to translate this vaccination approach to human AD patients generated iatrogenic meningoencephalitis in about 6% of individuals [11]. Mice can be induced to undergo similar reactions when overexpressing interferon (IFN)-γ [12], suggesting that immune responses tilted in favor of Th1 responses foster cell-mediated and/or inflammatory reactions to the vaccination. There is a considerable elaboration of inflammatory index in all AD brains [13, 14], including the activation of microglia;

apparently, this neuroinflammation is fostered by Aβ itself [15, 16]. It is possible that these proinflammatory actions of Aβ create conditions unfavorable for the development of humoral immune responses.

IL-12 family cytokines are heterodimeric proteins which include IL-12 and IL-23. IL-12 is composed of p40 and p35 subunits, and IL-23 is composed of the same p40 subunit together with a unique p19 subunit [17]. IL-12 plays a critical role in the differentiation of $CD4^+$ Th1 lymphocytes. These Th1 lymphocytes stimulate cell-mediated immune responses important in clearing pathogens, including viruses and bacteria. Th1 lymphocytes produce IFN-γ which activates cells of the innate immune system and contributes to the clearance of these pathogens. IL-23 stimulates the differentiation of a unique set of $CD4^+$ T lymphocytes. These cells are characterized by the production of the cytokine IL-17 and are termed as Th17 lymphocytes [18]. Recent studies indicated that mice genetically ablated of the p19 subunit of IL-23 are resistant to the development of experimental autoimmune encephalomyelitis (EAE), whereas mice lacking the p35 subunit of IL-12 showed similar or more severe EAE than that observed in wild-type animals [19–21]. It thus appears that IL-12 and IL-23 each play important yet distinct roles in the development of immune responses that tend towards cell-mediated modalities which can include inflammation. Thus, suppressing the production of these cytokines may be effective in the treatment of inflammatory diseases.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor family of transcriptional activators. Three PPAR subtypes exist (PPAR-α, PPAR-γ, and PPAR-β/δ), each exhibiting distinct patterns of tissue expression and ligand specificities [22]. The role of PPAR-γ in modulating adipogenesis and glucose metabolism is well established. Thiazolidinediones are PPAR-γ agonists that are currently used extensively in the treatment of type II diabetes. More recently, the role of PPAR-γ agonists in modulating immune responses, including immune responses in the CNS, has become appreciated. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce AD risk and ameliorate microglial reactivity in AD brains [23]. Since NSAIDs bind to and activate PPAR-γ, resulting in reduced expression of proinflammatory genes, this receptor may mediate the observed anti-inflammatory effects of NSAIDs in AD brain. In addition, it has been demonstrated that the PPAR-γ agonists, pioglitazone and ibuprofen, reduced glial inflammation and $A\beta_{1\text{-}42}$ levels in APPV717I transgenic mice [24]. Collectively, these studies suggest that PPAR-γ agonists may be effective in the treatment of neurodegenerative diseases, including AD.

Pattern recognition receptors termed as Toll-like receptors (TLRs) play a critical role in the innate immune response to pathogen-associated molecular patterns (PAMPs) present in viruses, bacteria, and fungi [25]. They may also contribute to neuroinflammation triggered by endogenous ligands [25] or simply overexpression of the receptors alone [26]. A series of eleven TLRs have been identified in mice and humans, each capable of binding distinct PAMPs. The PAMP lipopolysaccharide (LPS) binds to TLR4 in association with another pattern recognition receptor termed as CD14. With the exception of TLR3, ligand binding to TLRs stimulates recruitment of the adaptor molecule MyD88, activating a well-defined signal transduction pathway that culminates in activation of the transcription factor NF-κB, which elevates expression of a variety of proinflammatory genes [27]. TLR4 has been suggested to play a role in regulating the pathogenesis of AD in humans [28, 29] and in animal models of AD [30]. This suggests that agents capable of altering MyD88-dependent TLR signaling may modulate the development of AD.

The current studies indicate that the PPAR-γ agonist 15d-PGJ_2 suppresses the production of IL-12 and IL-23 by Aβ plus LPS-stimulated microglia. These cytokines play critical roles in Th1 and Th17 cell differentiation. These studies could have important implications concerning Aβ immunization as therapy for AD. In addition, we demonstrate that 15d-PGJ_2 inhibits Aβ plus LPS stimulation of MyD88, CD14, and TLR2 expression by microglia, suggesting that this cyclopentenone prostaglandin inhibits MyD88-dependent signaling. This provides a potential mechanism by which the PPAR-γ agonist 15d-PGJ_2 modulates the expression of proinflammatory cytokines.

2. MATERIALS AND METHODS

2.1. Reagents

15d-PGJ_2 was obtained from Cayman Chemical Company (Ann Arbor, Mich, USA). Lipopolysaccharide and lectin, *Griffonia simplicifolia,* were obtained from Sigma (St. Louis, Mo, USA). $A\beta_{1\text{-}42}$ was obtained from AnaSpec, Inc. (San Jose, Calif, USA). DMEM media, glutamine, trypsin, and antibiotics used for tissue culture were obtained from BioWhittaker (Walkersville, Md, USA). OPI medium supplement was obtained from Sigma. Fetal bovine serum (FBS) was obtained from Hyclone (Logan, Utah, USA). GM-CSF was obtained from BD Pharmingen (San Diego, Calif, USA). N-2 supplement was obtained from Gibco Invitrogen Corporation (Carlsbad, Calif, USA). Glial fibrillary acidic protein (GFAP) was obtained from Dako (Carpinteria, Calif, USA). C57BL/6 mice were obtained from Harlan (Indianapolis, Ind, USA) and bred in house.

2.2. Cell culture

Primary mouse microglia cultures were obtained through a modification of the McCarthy and deVellis protocol [31]. Briefly, cerebral cortices from 1–3 day-old C57BL/6 mice were excised, meninges removed, and cortices minced into small pieces. Cells were separated by trypsinization followed by trituration of cortical tissue. The cell suspension was filtered through a 70 μm cell strainer to remove debris. Cells were centrifuged at 153 $\times$ g for 5minutes at 4°C, resuspended in DMEM medium containing 10% FBS, 1.4 mm L-glutamine, 100 U/mL penicillin, 0.1mg/mL streptomycin, OPI medium supplement, and 0.5 ng/mL recombinant mouse GM-CSF, and plated into tissue culture flasks. Cells were allowed to grow to confluency (7–10 days) at 37°C/5%

CO_2. Flasks were then shaken overnight (200 rpm at 37°C) in a temperature-controlled shaker to loosen microglia and oligodendrocytes from the more adherent astrocytes. These less adherent cells were plated for 2-3 hours and then lightly shaken to separate oligodendrocytes from the more adherent microglia. Microglia were seeded in 24-well plates or 6-well plates and incubated overnight at 37°C/5% CO_2. After overnight incubation, cells were treated with 15d-PGJ_2 for 1 hour in the serum free medium with N-2 supplement, and then stimulated with A$\beta_{1\text{-}42}$ and/or LPS for 6 or 24 hours. A$\beta_{1\text{-}42}$ peptides were dissolved in DMSO to prepare a 5 mM stock solution, which was aliquoted and stored at −80°C. A$\beta_{1\text{-}42}$ stock solution was diluted with culture medium to a concentration of 0.1 mM, and set at room temperature for 12–18 hours before use. The final applied concentration of DMSO from A$\beta_{1\text{-}42}$ was ≤0.2%. After the 24-hour stimulation, tissue culture supernatants were collected for enzyme-linked immunosorbent assay (ELISA), and cell viability was analyzed; 6 hours after stimulation, total RNA was collected for real-time quantitative RT-PCR (qRT-PCR) analysis. The purity of microglia cultures was greater than 95% as determined by immunohistochemical staining with the lectin, *Griffonia simplicifolia*. Astrocyte contamination of the microglial cultures was assessed by immunohistochemical staining with anti-GFAP.

2.3. Cell viability assay

Cell viability was determined by MTT reduction assay as described previously [32]. Optical densities were determined using a Spectromax 190 microplate reader (Molecular Devices, Sunnyvale, Calif, USA) at 570 nm. Results were reported as percent viability relative to untreated cultures.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Cytokine (IL-12p40, IL-12p70, and IL-1β) levels in tissue culture media were determined by ELISA as described by the manufacturer (OptEIA Sets, Pharmingen, San Diego, Calif, USA). Cytokine IL-23 (p19/p40) levels in tissue culture media were determined by ELISA as described by the manufacturer (eBioscience, San Diego, Calif, USA). Optical densities were determined using a Spectromax 190 microplate reader (Molecular Devices, Sunnyvale, Calif, USA) at 450 nm. Cytokine concentrations in media were determined from standards containing known concentrations of the proteins.

2.5. RNA isolation and cDNA synthesis

Total RNA was isolated from microglia using the RNeasy Mini Kit (Qiagen Sciences, Md, USA). RNA samples were treated with DNAse1 (Invitrogen, Carlsbad, Calif, USA) to remove any traces of contaminating DNA. The reverse transcription (RT) reactions were carried out using an iScript cDNA synthesis kit (Bio-Rad, Hercules, Calif, USA) according to the manufacturer's instructions.

2.6. Real-time quantitative RT-PCR assay

IL-12p40, IL-12p35, IL-23p19, IL-1β, CD14, MyD88, TLR2, and TLR4 mRNAs were quantified by real-time PCR using an iCycler IQ multicolor real-time PCR detection system (Bio-Rad). All primers and TaqMan MGB probes (FAMdye-labeled) were designed and synthesized by Applied Biosystems (Foster City, Calif, USA). The real-time PCR reactions were performed in a total volume of 25 μL using an iCycler kit (Bio-Rad). The levels of IL-12p40, IL-12p35, IL-23p19, IL-1β, CD14, MyD88, TLR2, and TLR4 mRNA expression in primary microglia were calculated after normalizing cycle thresholds against the "housekeeping" gene GAPDH, and are presented as the fold induction value ($2^{-\Delta\Delta Ct}$) relative to LPS-stimulated microglia.

2.7. Statistics

Data were analyzed by one-way ANOVA followed by a Bonferroni posthoc test to determine the significance of difference.

3. RESULTS

3.1. Effects of 15d-PGJ_2 on IL-1β production by β-amyloid plus LPS-stimulated microglia

A variety of studies suggest that the inflammatory cytokine IL-1β plays a significant role in modulating the pathogenesis of AD [33]. In the present study, we investigated whether A$\beta_{1\text{-}42}$ plus a low dose of LPS could induce IL-1β production by primary mouse microglial cells. Our results showed that A$\beta_{1\text{-}42}$ alone did not induce microglia production of IL-1β protein (Figure 1(a)) and IL-1β mRNA (Figure 1(b)). LPS (10 ng/mL) alone stimulated microglial production of IL-1β protein and mRNA, while a combination of A$\beta_{1\text{-}42}$ and LPS synergistically induced the expression of IL-1β protein and mRNA. Interestingly, the PPAR-γ agonist 15d-PGJ_2 strongly suppressed induction of IL-1β in A$\beta_{1\text{-}42}$ plus LPS-stimulated primary microglial cells. The PPAR-γ agonist did not decrease the viability of these microglial cells compared to cells treated with A$\beta_{1\text{-}42}$ plus LPS as determined by MTT analysis (data not shown). Therefore, the effects of 15d-PGJ_2 on the production of IL-1β were not due to effects on cell viability. These studies suggest that 15d-PGJ_2 may suppress the production of IL-1β, an inflammation-related cytokine associated with the pathogenesis of AD.

3.2. Effects of 15d-PGJ_2 on IL-12 family cytokines by β-amyloid plus LPS-stimulated microglia

IL-12 family cytokines are believed to contribute to the differentiation of Th1 and Th17 cells. A$\beta_{1\text{-}42}$ alone had little or no effect on the production of IL-12 family cytokines by microglia. LPS (10 ng/mL) stimulated microglia to secrete IL-12 family cytokines including IL-12p40 (Figure 2(a)), IL-12p70 (Figure 2(b)), and IL-23 (Figure 2(c)). In the context of this inflammatory priming, A$\beta_{1\text{-}42}$ further increased microglial production of each of these IL-12 family proteins significantly. Furthermore, the PPAR-γ agonist 15d-PGJ_2

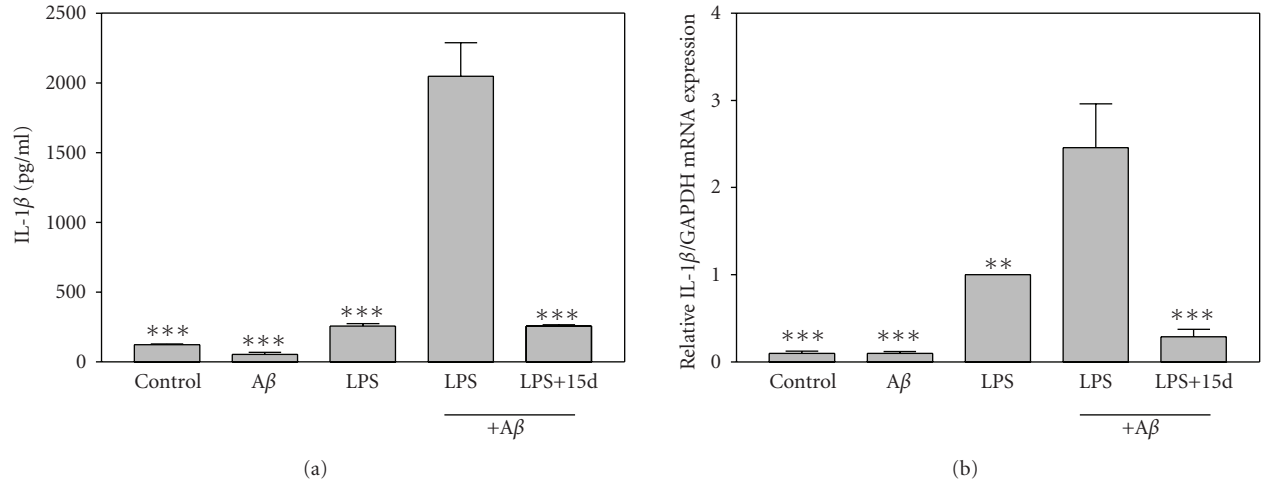


Figure 1: *15d-PGJ$_2$ inhibits IL-1β expression by Aβ$_{1\text{-}42}$ plus LPS-activated microglia.* (a) Primary mouse microglial cells were pretreated for 1 hour with 15d-PGJ$_2$ (2.5 μM). Aβ$_{1\text{-}42}$ (5 μM), LPS (10 ng/mL), or Aβ$_{1\text{-}42}$ (5 μM) plus LPS (10 ng/mL) was added as indicated, and 24 hours later, the concentration of IL-1β in the culture medium was determined. Values represent the mean ± s.e.m for a representative experiment run in triplicate. At least three independent experiments were conducted. (b) Cells were pretreated for 1 hour with 15d-PGJ$_2$ (2.5 μM). Aβ$_{1\text{-}42}$ (10 μM), LPS (5 ng/mL), or Aβ$_{1\text{-}42}$ (10 μM) plus LPS (5 ng/mL) was added as indicated, and 6 hours later, total RNA was isolated. IL-1β mRNA levels were determined by real-time quantitative RT-PCR. Results are expressed as fold inductions in GAPDH normalized mRNA values versus levels in LPS-treated cells. Values are mean ± s.e.m of six samples derived from three independent experiments, with each experiment performed in duplicate. $^{**}P < .01$ and $^{***}P < .001$ versus Aβ$_{1\text{-}42}$ + LPS-treated cultures.

significantly suppressed the expression of these IL-12 family proteins.

3.3. Effects of 15d-PGJ$_2$ on expression of IL-12 family cytokine subunit mRNAs by β-amyloid plus LPS-stimulated microglia

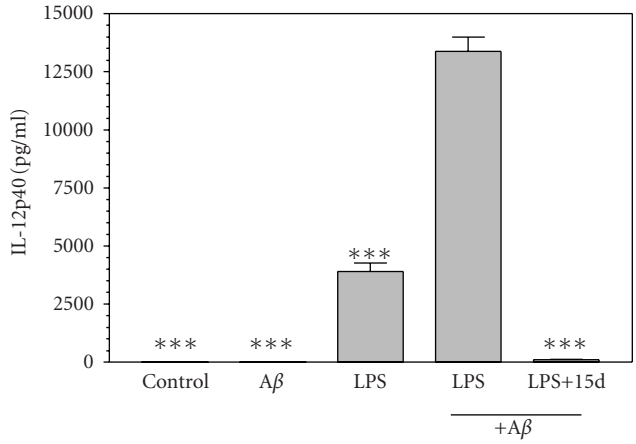
Aβ$_{1\text{-}42}$ alone had little or no effect on stimulating the expression of IL-12 family cytokine subunit mRNAs including IL-12p35 (Figure 3(a)), IL-12p40 (Figure 3(b)), and IL-23p19 (Figure 3(c)). Low doses of LPS (5 ng/mL) alone slightly induced the expression of these mRNAs. However, Aβ$_{1\text{-}42}$ in combination with LPS elicited significantly higher levels of IL-12 family subunit mRNAs compared to microglia stimulated with LPS alone. Pretreatment with 15d-PGJ$_2$ significantly suppressed the expression of IL-12 family subunit mRNAs. Thus, 15d-PGJ$_2$ inhibits the expression of IL-12 family cytokines and the mRNAs that encode these proteins. IL-12 and IL-23 play critical roles in the differentiation of Th1 and Th17 cells, which may contribute to the inflammatory events that resulted in cessation of clinical trials involving immunization of Aβ in the treatment of AD. Thus, cotreatment with 15d-PGJ$_2$ may increase the utility of Aβ immunotherapy for AD patients.

3.4. Effects of 15d-PGJ$_2$ on expression of Toll-like receptor signaling

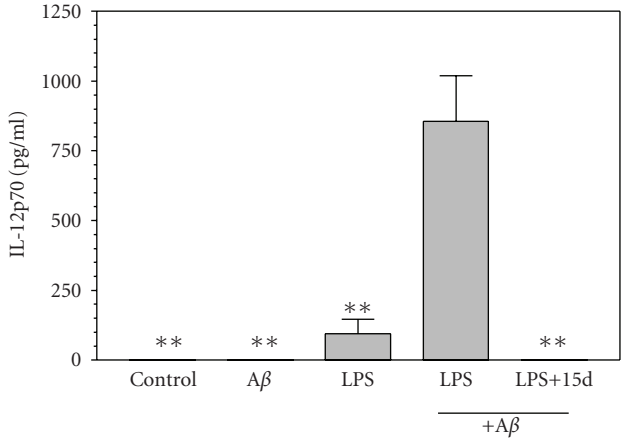
The MyD88-dependent TLR signaling pathway plays a critical role in modulating the response to PAMPs including LPS. We demonstrate that a combination of Aβ$_{1\text{-}42}$ plus LPS significantly induced the expression of CD14 relative to microglia treated with LPS alone (Figure 4(a)). In addition, Aβ$_{1\text{-}42}$ plus LPS also trended towards inducing MyD88 expression relative to each stimulus alone (Figure 4(b)). CD14 and MyD88 are critical intermediates in MyD88-dependent signaling. As we have demonstrated previously, LPS does not significantly induce the expression of TLR4, but does induce the expression of TLR2 [34]. Similarly, Aβ$_{1\text{-}42}$ in combination with LPS did not induce microglial expression of TLR4 (Figure 4(c)), but did induce the expression of TLR2 (Figure 4(d)). Interestingly, 15d-PGJ$_2$ inhibited Aβ$_{1\text{-}42}$ plus LPS induction of MyD88, CD14, and TLR2 mRNA expression in microglia. These studies suggest that 15d-PGJ2 may suppress inflammatory responses stimulated by Aβ$_{1\text{-}42}$ plus LPS by inhibiting MyD88-dependent TLR signaling.

4. DISCUSSION

AD currently affects over 200 million people worldwide. Disease incidence is expected to increase as the population ages, and the socioeconomic impact of AD is staggering. The disease is characterized in part by the presence of neuritic plaques which contain accumulations of insoluble Aβ. Vaccination with Aβ synthetic peptides in animal models of AD suggested that such immunizations may be effective in the treatment of AD in humans. For example, Aβ immunization of APP transgenic mice decreases the density and number of Aβ deposits in the brains of these mice. Decreased Aβ deposits in these mice are associated with decreased neuritic dystrophy and gliosis [7]. Intranasal administration of Aβ engenders humoral responses that include immunoglobulin isotypes consistent with a Th2 response, and this is associated with increased clearance of amyloid [35]. Significantly, active immunization against Aβ in APP transgenic mice decreases memory deficits in



(a)



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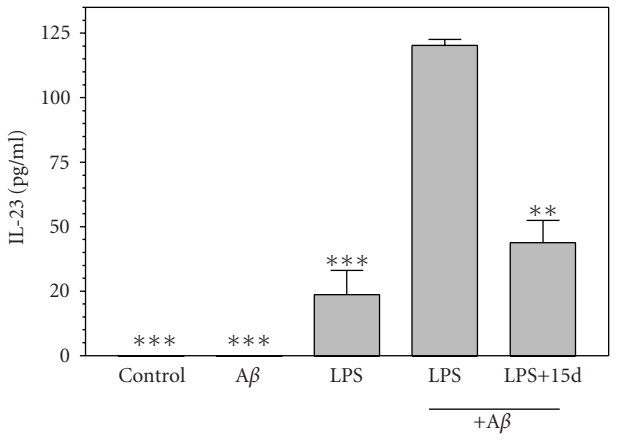
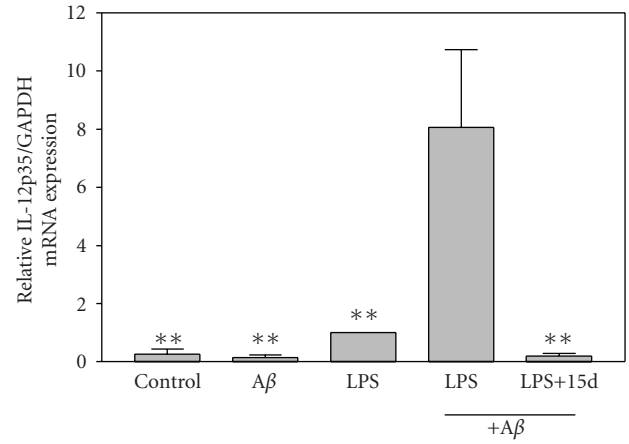
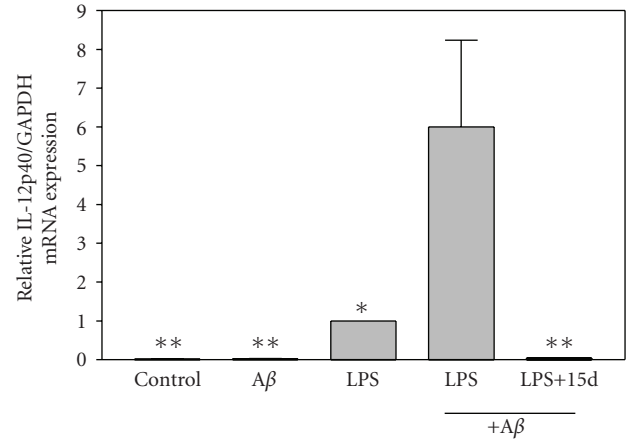


(c)

Figure 2: *15d-PGJ$_2$ inhibits IL-12 family cytokines production by A$\beta_{1\text{-}42}$ plus LPS-activated microglia*. Cells were pretreated for 1 hour with 15d-PGJ$_2$ (2.5 μM). A$\beta_{1\text{-}42}$ (5 μM), LPS (10 ng/mL), or A$\beta_{1\text{-}42}$ (5 μM) plus LPS (10 ng/mL) was added as indicated, and 24 hours later, the concentration of IL-12p40 (a), IL-12p70 (p35/p40) (b), and IL-23 (p19/p40) (c) in the culture medium was determined. Values represent the mean ± s.e.m for a representative experiment run in triplicate. At least three independent experiments were conducted. $^{**}P < .01$ and $^{***}P < .001$ versus A$\beta_{1\text{-}42}$ + LPS-treated cultures.



(a)



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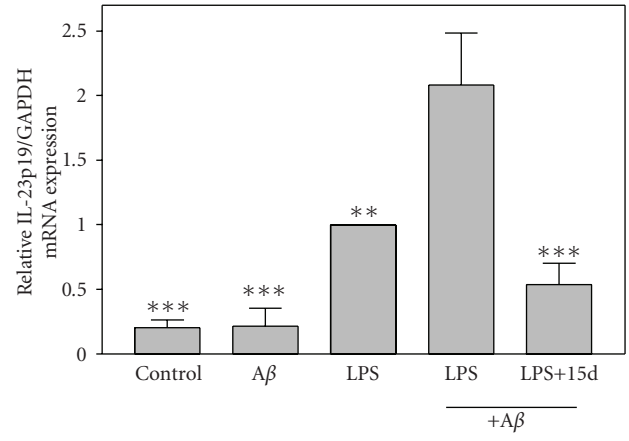

(c)

Figure 3: *15d-PGJ$_2$ inhibits microglial mRNA expression of IL-12p40, IL-12p35, and IL-23p19 induced by A$\beta_{1\text{-}42}$ plus LPS*. Cells were pretreated for 1 hour with 15d-PGJ$_2$ (2.5 μM). A$\beta_{1\text{-}42}$ (10 μM), LPS (5 ng/mL), or A$\beta_{1\text{-}42}$ (10 μM) plus LPS (5 ng/mL) was added as indicated, and 6 hours later, total RNA was isolated. IL-12p35 (a), IL-12p40 (b), and IL-23p19 (c) mRNA levels were determined by real-time quantitative RT-PCR. Results are expressed as fold inductions in GAPDH normalized mRNA values versus levels in LPS-treated cells. Values are mean ± s.e.m of six samples derived from three independent experiments, with each experiment performed in duplicate. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ versus A$\beta_{1\text{-}42}$ + LPS-treated cultures.

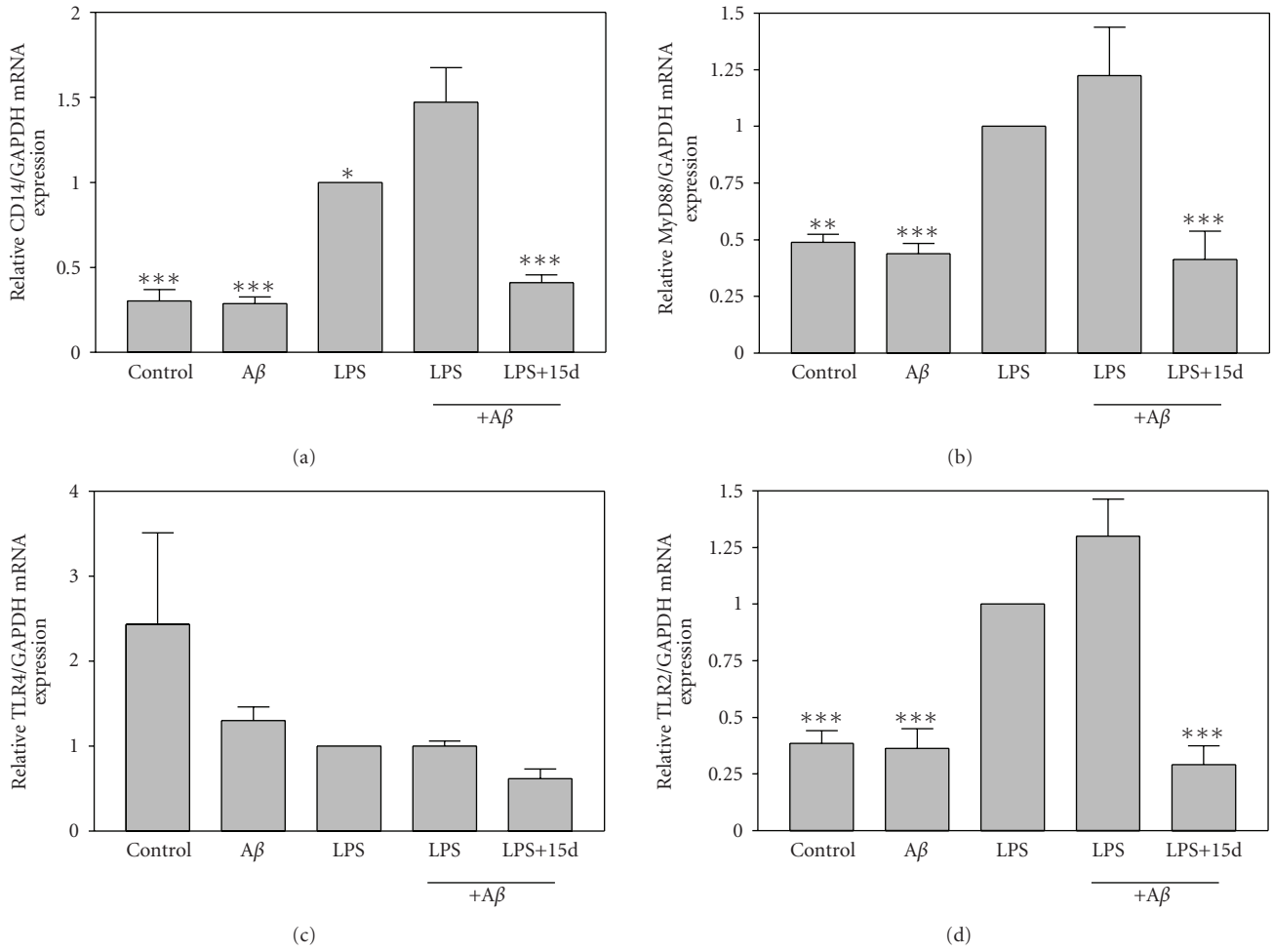

Figure 4: *The effects of 15d-PGJ$_2$ on microglial mRNA expression of CD14, MyD88, TLR4, and TLR2 induced by A$\beta_{1\text{-}42}$ plus LPS*. Cells were pretreated for 1 hour with 15d-PGJ$_2$ (2.5 μM). A$\beta_{1\text{-}42}$ (10 μM), LPS (5 ng/mL), or A$\beta_{1\text{-}42}$ (10 μM) plus LPS (5 ng/mL) was added as indicated, and 6 hours later, total RNA was isolated. CD14 (a), MyD88 (b), TLR4 (c), and TLR2 (d) mRNA levels were determined by real-time quantitative RT-PCR. Results are expressed as fold inductions in GAPDH normalized mRNA values versus levels in LPS-treated cells. Values are mean ± s.e.m of six samples derived from three independent experiments, with each experiment performed in duplicate. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$ versus A$\beta_{1\text{-}42}$ + LPS-treated cultures.

these mice [6, 35, 36]. Interestingly, passive administration of monoclonal antibodies specific for Aβ peptides is also effective in clearing Aβ and improving memory deficits in APP transgenic mice [37, 38]. This suggests that Aβ-specific antibodies produced following immunization are the critical factor mediating AD-like pathology in these animal models of AD. Three potential mechanisms have been suggested that may determine how anti-Aβ antibodies reduce Aβ deposits in the brains of APP transgenic mice. Aβ antibodies (1) may directly dissolve Aβ deposits, (2) may stimulate Fc-receptor-mediated phagocytosis of Aβ by microglia, and/or (3) may stimulate Aβ efflux from the brain to the plasma [3].

Animal studies indicating that Aβ immunization of APP transgenic mice reduced plaque burden in mice stimulated human clinical trials designed to evaluate the clinical efficacy of Aβ immunization in the treatment of AD. Small-scale phase I trials indicated apparent safety of Aβ immunization, and demonstrated that the majority of mild to moderate AD patients immunized in these studies produced anti-Aβ antibodies [39, 40]. However, subsequent larger-scale phase II clinical trials were halted when approximately 6% of Aβ immunized patients developed meningoencephalitis [11]. Postmortem evaluation indicated that Aβ immunization resulted in decreased plaque burden in the cortex of treated patients [41–43], and these brain regions were associated with abundant Aβ immunoreactive microglia, suggesting that these cells were involved in the removal of Aβ [43]. Interestingly, although anti-Aβ antibodies are believed to contribute to the reduction in Aβ plaques in AD patients, antibodies titers did not correlate with the development of meningoencephalitis [11, 40]. Several studies suggest that T cell responses to Aβ may have stimulated the development of meningoencephalitis in immunized AD patients [44–46]. Furthermore, a higher T cell reactivity to Aβ has been observed in some elderly and AD patients not immunized with Aβ. This suggests that the elderly population and AD

patients may exhibit increased susceptibility to the development of meningoencephalitis following Aβ vaccination [46]. Cases of meningoencephalitis were associated with increased infiltration of both $CD4^+$ and $CD8^+$ T cells [42]. However, it is generally believed that $CD4^+$ Th1 cells triggered the development of meningoencephalitis following Aβ immunization [3]. The potential role of the recently described $CD4^+$Th17 cells in the production of meningoencephalitis has not been evaluated. However, studies indicating that these cells play a critical role in the development of MS and other autoimmune disorders suggest that these cells may also play a role in the development of meningoencephalitis in Aβ immunized AD patients.

Our current studies indicate that the PPAR-γ agonist 15d-PGJ_2 inhibits microglial production of IL-12 and IL-23, which play critical roles in the differentiation of Th1 and Th17 cells, respectively. This suggests that 15d-PGJ_2 could potentially increase the efficacy and safety of Aβ immunization of AD patients by decreasing or abolishing the development of meningoencephalitis in these patients. Epidemiological studies indicated that nonsteroidal anti-inflammatory drugs (NSAIDs) reduced the risk of AD. Some NSAIDs are capable of activating PPAR-γ, suggesting that these drugs may modulate development of AD through activation of this receptor [47, 48]. The role of PPAR-γ in modulating AD is supported by studies indicating that ibuprofen reduced $A\beta_{1\text{-}42}$ levels in APP transgenic mouse models of AD, while low levels of the thiazolidinedione pioglitazone stimulated a slight yet statistically insignificant reduction of $A\beta_{1\text{-}42}$ levels in these mice [49]. In a later study, higher levels of pioglitazone decreased astrocyte and microglial activation and Aβ plaque burden in APP transgenic mice [24]. Similarly, the thiazolidinedione rosiglitazone also decreased $A\beta_{1\text{-}42}$ levels in animal models of AD [50]. Collectively, these studies support a role for PPAR-γ in modulating AD pathology. Studies indicate that PPAR-γ activation suppresses expression of β-site of APP cleaving enzyme (BACE)-1, suggesting that PPAR-γ agonists may modulate AD pathogenesis at least in part by altering Aβ homeostasis [51]. Importantly, recent clinical studies demonstrated that rosiglitazone was effective in improving cognition in a subset of AD patients [52, 53]. The fact that rosiglitazone exhibits poor blood-brain barrier penetration suggests that this PPAR-γ agonist may act in the periphery and not directly in the CNS.

We and others have previously demonstrated that PPAR-γ agonists are capable of suppressing the activation of NF-κB, which is a potent transcriptional activator of a variety of genes encoding proinflammatory molecules. MyD88-dependent signaling results in the activation of NF-κB. In the current studies, we demonstrate that the PPAR-γ agonist 15d-PGJ_2 suppressed microglial expression of MyD88 and CD14 which are critical intermediates in MyD88-dependent TLR signaling. In addition, we demonstrate that 15d-PGJ_2 inhibits microglial expression of IL-1β, a cytokine believed to contribute to AD pathogenesis [33]. Thus, PPAR-γ agonists may act as general suppressors of classical activation of microglia. Since classically activated microglia produce neurotoxic molecules, suppression of microglial activation may protect against AD. However, it should also be noted that some form of microglial activation may help remove Aβ plaques from AD brains through phagocytosis. In addition, TLR and CD14 molecules have been suggested to contribute to—or alternatively protect against—the development of AD [30, 54, 55]. It clearly appears that microglia and microglial products modulate AD through a series of complex and potentially conflicting mechanisms.

In summary, we have demonstrated that the PPAR-γ agonist 15d-PGJ_2 inhibits production of IL-12 and IL-23 by Aβ plus LPS-activated microglia. These cytokines regulate the differentiation of Th1 and Th17 cells, which may limit the efficacy of Aβ immunotherapy for the treatment of AD. Furthermore, we demonstrate that 15d-PGJ_2 inhibits the production of IL-1β by microglia, a cytokine known to play a role in AD pathogenesis. Finally, we demonstrate that 15d-PGJ_2 inhibits the expression of MyD88-dependent signaling intermediates, suggesting a mechanism by which this PPAR-γ agonist may suppress inflammation. Collectively, these studies contribute to the body of evidence indicating that PPAR-γ agonists may be effective in the treatment of AD.

ACKNOWLEDGMENTS

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REFERENCES

[1] N. Abbas, I. Bednar, E. Mix, et al., "Up-regulation of the inflammatory cytokines IFN-γ and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP_{SWE} transgenic mice," *Journal of Neuroimmunology*, vol. 126, no. 1-2, pp. 50–57, 2002.

[2] D. Schenk, "Amyloid-β immunotherapy for Alzheimer's disease: the end of the beginning," *Nature Reviews Neuroscience*, vol. 3, no. 10, pp. 824–828, 2002.

[3] H. L. Weiner and D. Frenkel, "Immunology and immunotherapy of Alzheimer's disease," *Nature Reviews Immunology*, vol. 6, no. 5, pp. 404–416, 2006.

[4] D. Games, M. Buttini, D. Kobayashi, D. Schenk, and P. Seubert, "Mice as models: transgenic approaches and Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 9, supplement 3, pp. 133–149, 2006.

[5] D. Morgan, "Immunotherapy for Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 9, supplement 3, pp. 425–432, 2006.

[6] C. Janus, J. Pearson, J. McLaurin, et al., "Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 979–982, 2000.

[7] D. Schenk, R. Barbour, W. Dunn, et al., "Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse," *Nature*, vol. 400, no. 6740, pp. 173–177, 1999.

[8] D. M. Wilcock, M. N. Gordon, K. E. Ugen, et al., "Number of Aβ inoculations in APP+PS1 transgenic mice influences antibody titers, microglial activation, and congophilic plaque levels," *DNA and Cell Biology*, vol. 20, no. 11, pp. 731–736, 2001.

[9] M. H. Mohajeri, K. Saini, J. G. Schultz, M. A. Wollmer, C. Hock, and R. M. Nitsch, "Passive immunization against

β-amyloid peptide protects central nervous system (CNS) neurons from increased vulnerability associated with an Alzheimer's disease-causing mutation," *Journal of Biological Chemistry*, vol. 277, no. 36, pp. 33012–33017, 2002.

[10] D. M. Wilcock, A. Rojiani, A. Rosenthal, et al., "Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition," *Journal of Neuroscience*, vol. 24, no. 27, pp. 6144–6151, 2004.

[11] J.-M. Orgogozo, S. Gilman, J.-F. Dartigues, et al., "Subacute meningoencephalitis in a subset of patients with AD after Aβ42 immunization," *Neurology*, vol. 61, no. 1, pp. 46–54, 2003.

[12] A. Monsonego, J. Imitola, S. Petrovic, et al., "Aβ-induced meningoencephalitis is IFN-γ-dependent and is associated with T cell-dependent clearance of Aβ in a mouse model of Alzheimer's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 13, pp. 5048–5053, 2006.

[13] M. T. Heneka and M. K. O'Banion, "Inflammatory processes in Alzheimer's disease," *Journal of Neuroimmunology*, vol. 184, no. 1-2, pp. 69–91, 2007.

[14] J. C. S. Breitner, "The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease," *Annual Review of Medicine*, vol. 47, pp. 401–411, 1996.

[15] L. Meda, M. A. Cassatella, G. I. Szendrei, et al., "Activation of microglial cells by β-amyloid protein and interferon-γ," *Nature*, vol. 374, no. 6523, pp. 647–650, 1995.

[16] C. A. Saura, G. Chen, S. Malkani, et al., "Conditional inactivation of presenilin 1 prevents amyloid accumulation and temporarily rescues contextual and spatial working memory impairments in amyloid precursor protein transgenic mice," *Journal of Neuroscience*, vol. 25, no. 29, pp. 6755–6764, 2005.

[17] B. Oppmann, R. Lesley, B. Blom, et al., "Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12," *Immunity*, vol. 13, no. 5, pp. 715–725, 2000.

[18] C. L. Langrish, B. S. McKenzie, N. J. Wilson, R. de Waal Malefyt, R. A. Kastelein, and D. J. Cua, "IL-12 and IL-23: master regulators of innate and adaptive immunity," *Immunological Reviews*, vol. 202, pp. 96–105, 2004.

[19] B. Becher, B. G. Durell, and R. J. Noelle, "Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12," *Journal of Clinical Investigation*, vol. 110, no. 4, pp. 493–497, 2002.

[20] D. J. Cua, J. Sherlock, Y. Chen, et al., "Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain," *Nature*, vol. 421, no. 6924, pp. 744–748, 2003.

[21] B. Gran, G.-X. Zhang, S. Yu, et al., "IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination," *Journal of Immunology*, vol. 169, no. 12, pp. 7104–7110, 2002.

[22] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[23] L. Gasparini, E. Ongini, and G. Wenk, "Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: old and new mechanisms of action," *Journal of Neurochemistry*, vol. 91, no. 3, pp. 521–536, 2004.

[24] M. T. Heneka, M. Sastre, L. Dumitrescu-Ozimek, et al., "Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV717I transgenic mice," *Brain*, vol. 128, no. 6, pp. 1442–1453, 2005.

[25] K. Takeda, T. Kaisho, and S. Akira, "Toll-like receptors," *Annual Review of Immunology*, vol. 21, pp. 335–376, 2003.

[26] N. Harii, C. J. Lewis, V. Vasko, et al., "Thyrocytes express a functional Toll-like receptor 3: overexpression can be induced by viral infection and reversed by phenylmethimazole and is associated with Hashimoto's autoimmune thyroiditis," *Molecular Endocrinology*, vol. 19, no. 5, pp. 1231–1250, 2005.

[27] T. Kielian, "Toll-like receptors in central nervous system glial inflammation and homeostasis," *Journal of Neuroscience Research*, vol. 83, no. 5, pp. 711–730, 2006.

[28] C. R. Balistreri, G. Candore, F. Listì, et al., "Role of TLR4 polymorphisms in inflammatory responses: implications for unsuccessful aging," *Annals of the New York Academy of Sciences*, vol. 1119, no. 1, pp. 203–207, 2007.

[29] S. Walter, M. Letiembre, Y. Liu, et al., "Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease," *Cellular Physiology and Biochemistry*, vol. 20, no. 6, pp. 947–956, 2007.

[30] K. Tahara, H.-D. Kim, J.-J. Jin, J. A. Maxwell, L. Li, and K. Fukuchi, "Role of toll-like receptor signalling in Aβ uptake and clearance," *Brain*, vol. 129, no. 11, pp. 3006–3019, 2006.

[31] K. D. McCarthy and J. de Vellis, "Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue," *Journal of Cell Biology*, vol. 85, no. 3, pp. 890–902, 1980.

[32] P. D. Drew and J. A. Chavis, "The cyclopentone prostaglandin 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 represses nitric oxide, TNF-α, and IL-12 production by microglial cells," *Journal of Neuroimmunology*, vol. 115, no. 1-2, pp. 28–35, 2001.

[33] R. E. Mrak and W. S. T. Griffin, "Common inflammatory mechanisms in Lewy body disease and Alzheimer disease," *Journal of Neuropathology & Experimental Neurology*, vol. 66, no. 8, pp. 683–686, 2007.

[34] J. Xu and P. D. Drew, "Peroxisome proliferator-activated receptor-γ agonists suppress the production of IL-12 family cytokines by activated glia," *Journal of Immunology*, vol. 178, no. 3, pp. 1904–1913, 2007.

[35] M. Maier, T. J. Seabrook, N. D. Lazo, et al., "Short amyloid-β (Aβ) immunogens reduce cerebral Aβ load and learning deficits in an Alzheimer's disease mouse model in the absence of an Aβ-specific cellular immune response," *Journal of Neuroscience*, vol. 26, no. 18, pp. 4717–4728, 2006.

[36] D. Morgan, D. M. Diamond, P. E. Gottschall, et al., "Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 982–985, 2000, Erratum in *Nature*, vol. 412, no. 6847, p. 660, 2001.

[37] F. Bard, C. Cannon, R. Barbour, et al., "Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease," *Nature Medicine*, vol. 6, no. 8, pp. 916–919, 2000.

[38] R. B. DeMattos, K. R. Bales, D. J. Cummins, J.-C. Dodart, S. M. Paul, and D. M. Holtzman, "Peripheral anti-Aβ antibody alters CNS and plasma Aβ clearance and decreases brain Aβ burden in a mouse model of Alzheimer's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 15, pp. 8850–8855, 2001.

[39] A. J. Bayer, R. Bullock, R. W. Jones, et al., "Evaluation of the safety and immunogenicity of synthetic Aβ42 (AN1792) in patients with AD," *Neurology*, vol. 64, no. 1, pp. 94–101, 2005.

[40] S. Gilman, M. Koller, R. S. Black, et al., "Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial," *Neurology*, vol. 64, no. 9, pp. 1553–1562, 2005.

[41] J. A. R. Nicolll, D. Wilkinson, C. Holmes, P. Steart, H. Markham, and R. O. Weller, "Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: a case report," *Nature Medicine*, vol. 9, no. 4, pp. 448–452, 2003.

[42] I. Ferrer, M. Boada Rovira, M. L. Sánchez Guerra, M. J. Rey, and F. Costa-Jussá, "Neuropathology and pathogenesis of encephalitis following amyloid-β immunization in Alzheimer's disease," *Brain Pathology*, vol. 14, no. 1, pp. 11–20, 2004.

[43] E. Masliah, L. Hansen, A. Adame, et al., "Aβ vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease," *Neurology*, vol. 64, no. 1, pp. 129–131, 2005.

[44] H. L. Weiner and D. J. Selkoe, "Inflammation and therapeutic vaccination in CNS diseases," *Nature*, vol. 420, no. 6917, pp. 879–884, 2002.

[45] A. Monsonego and H. L. Weiner, "Immunotherapeutic approaches to Alzheimer's disease," *Science*, vol. 302, no. 5646, pp. 834–838, 2003.

[46] A. Monsonego, V. Zota, A. Karni, et al., "Increased T cell reactivity to amyloid β protein in older humans and patients with Alzheimer disease," *Journal of Clinical Investigation*, vol. 112, no. 3, pp. 415–422, 2003.

[47] T. Kielian and P. D. Drew, "Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation," *Journal of Neuroscience Research*, vol. 71, no. 3, pp. 315–325, 2003.

[48] M. T. Heneka, G. E. Landreth, and D. L. Feinstein, "Role of peroxisome proliferator-activated receptor-γ in Alzheimer's disease," *Annals of Neurology*, vol. 49, no. 2, p. 276, 2001.

[49] Q. Yan, J. Zhang, H. Liu, et al., "Anti-inflammatory drug therapy alters β-amyloid processing and deposition in an animal model of Alzheimer's disease," *Journal of Neuroscience*, vol. 23, no. 20, pp. 7504–7509, 2003.

[50] W. A. Pedersen, P. J. McMillan, J. J. Kulstad, J. B. Leverenz, S. Craft, and G. R. Haynatzki, "Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice," *Experimental Neurology*, vol. 199, no. 2, pp. 265–273, 2006.

[51] M. Sastre, I. Dewachter, G. E. Landreth, et al., "Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-γ agonists modulate immunostimulated processing of amyloid precursor protein through regulation of β-secretase," *Journal of Neuroscience*, vol. 23, no. 30, pp. 9796–9804, 2003.

[52] G. S. Watson, B. A. Cholerton, M. A. Reger, et al., "Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study," *American Journal of Geriatric Psychiatry*, vol. 13, no. 11, pp. 950–958, 2005.

[53] M. E. Risner, A. M. Saunders, J. F. B. Altman, et al., "Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease," *The Pharmacogenomics Journal*, vol. 6, no. 4, pp. 246–254, 2006.

[54] K. Fassbender, S. Walter, S. Kühl, et al., "The LPS receptor (CD14) links innate immunity with Alzheimer's disease," *The FASEB Journal*, vol. 18, no. 1, pp. 203–205, 2004.

[55] Y. Liu, S. Walter, M. Stagi, et al., "LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide," *Brain*, vol. 128, no. 8, pp. 1778–1789, 2005.