Peroxisome Proliferator-Activated Receptor Agonists as Potential Therapeutic Agents in Multiple Sclerosis

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Abstract: Peroxisome proliferator-activated receptors (PPARs) have been extensively studied for gene regulation in glucose and lipid metabolism. It has been recently implicated that PPARs regulate cellular proliferation and inflammatory responses; some agonists for PPARs ameliorate experimental autoimmune encephalomyelitis, a model of multiple sclerosis (MS) in humans. This article will outline current experimental evidence suggesting potential clinical benefits for patients with MS.

Key Words: PPAR, multiple sclerosis, experimental autoimmune encephalomyelitis, cytokine, inflammation.

INTRODUCTION

Multiple sclerosis (MS) is a major inflammatory and demyelinating disease of the central nervous system (CNS). It is commonly acquired when individuals are in approximately their 30s, and most patients experience the devastating effects of this disease for many years. The cause of MS remains unclear; however, the etiology is presumed to be autoimmunity, and many types of inflammatory agents, including pro-inflammatory cytokines, participate in the pathogenesis of MS. Furthermore, it was demonstrated that demyelinated axons are more susceptible to inflammatory mediators of activated macrophages and microglias, such as the tumor necrosis factor (TNF), nitric oxide (NO), toxic factors that directly activate glutamate receptors, and the tissue plasminogen activator [1-4]. The use of multiple drugs, including interferon-β (IFN-β), glatiramer acetate (GA), and mitoxantrone (MTX), has been approved for the treatment of MS. Recently, natalizumab was re-approved by the FDA. However, none of them are cure and better strategies need to be adopted for the successful treatment of MS.

Peroxisome proliferator-activated receptors (PPARs) constitute a family of transcription factors belonging to the nuclear receptor superfamily, which includes sex steroid, glucocorticoid, mineral corticoid, retinoic acid, thyroid, and vitamin D receptors, as well as a large number of orphan receptors having ligands that remain unidentified thus far. The role of PPARs in regulating the transcription of genes involved in glucose and lipid metabolism has been extensively characterized [5]. To date, three mammalian PPAR subtypes have been isolated: PPARα, PPARδ (also referred to as β), and PPARγ, and these exhibit differential tissues distributions and ligand specificities [6-8]. PPARα is expressed mainly in tissues involved in lipid oxidation, such as the liver, kidney, adrenal glands, cardiac muscle, and skeletal muscles [5,9]. PPARδ/β is found in many tissues but it shows highest expression in the gut, kidney, and heart [5]. In comparison to PAPRα and PPARδ/β, PPARγ has a more restricted tissue distribution; it shows high receptor levels in the fat tissues, spleen, colon, and macrophages and low (but detectable) receptor levels in the liver, skeletal muscle, and pancreas [10-12]. The PPAR family members play major roles in the regulation of lipid metabolism, glucose homeostasis, and inflammatory processes, making these transcription factors ideal targets for therapeutic strategies against these diseases. For example, synthetic thiazolidinediones, which are PPARγ ligands, are commonly prescribed for the treatment of type II diabetes. Interestingly, indomethacin and other nonsteroidal anti-inflammatory drugs (NSAIDs) that are used to treat inflammation are PPARγ ligands, and it was recently shown that some PPAR ligands have anti-inflammatory effects. Some of the agents for PPARs ameliorated colonic inflammation in a mouse model of inflammatory bowel disease (IBD) [13] and adjuvant-induced arthritis [14]. Recently, some PPAR ligands have been shown to be effective in the treatment of experimental autoimmune encephalomyelitis (EAE) as an animal model of MS.

This paper focuses on the new insights suggesting the role of PPARs in inflammation and then discusses the current experimental evidence in the case of PPARs and their ligands particularly in EAE and MS. These findings highlight their potential as therapeutic targets for the treatment of MS.

STRUCTURE OF PPARs

On sequence comparison, the PPAR subtypes (α, δ/β, and γ) showed a remarkable homology in the DNA binding and ligand binding domains, as shown in (Fig. **1**). PPARs consist of a variable N-terminal region that has a transcriptional activation function 1 domain (AF-1) responsible for the phosphorylation of PPAR and a DNA binding domain (DBD) which promotes the binding of PPAR to distinct regions of the peroxisome proliferator response element (PPRE) in the promoter region of the target genes. The ligand binding domain (LBD) includes the C-terminal region containing the transcriptional activation function 2 domain (AF-2). AF-2 is

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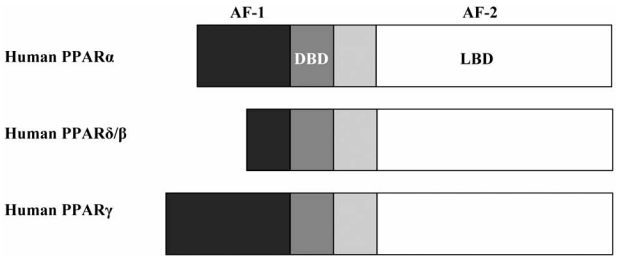

Fig. (1). Homology between the three PPAR subtypes. They have a variable N-terminal region that includes the transcriptional activation function 1 domain (AF-1) responsible for the phosphorylation of PPAR and the DNA binding domain (DBD), which promotes the binding of PPAR to distinct regions of the peroxisome proliferator response elements (PPRE) in the promoter region of the target genes. The ligand binding domain (LBD) includes the C-terminal region that contains the transcriptional activation function 2 domain (AF-2).

indispensable for ligand-dependent coactivator interaction and transactivation [15-17].

PAPR-MEDIATED SIGNALING

Understanding of the molecular mechanisms underlying the modulation of gene expression by PPARs and the ligands for these receptors has improved of late, although it is not entirely clear. PPARs are ligand-activated transcription factors. These receptors regulate gene expression through two mechanisms "transactivation" and "transrepression" (Fig. **2**). For transcriptional activation, PPARs need to heterodimerize with the 9-*cis* retinoic acid receptor (RXR); the transcriptional regulation of target genes by PPARs is achieved through the binding of these PPAR-RXR heterodimers to PPREs [18]. RXR also forms heterodimers with other members of the nuclear receptor superfamily, and these interactions influence the PPAR-regulated transcriptional activation because various RXR heterodimerization partners compete for RXR [18,19]. PPREs include an imperfect direct repeat of the consensus binding site for nuclear hormone receptors (AGGTCA) separated by one or two intervening nucleotides (direct repeats 1 and 2; DR-1 and DR-2) [20,21]. In the PPAR-RXR complex, PPAR binds to the 5′ extended half-site and RXR binds to the 3′ half site of the response elements, which are located in the promoter region of the PPAR target genes [20]. On activation by the ligand, the PPAR-RXR heterodimer recruits co-activator proteins that promote the initiation of transcription [22]. Due to these changes in the transcriptional activity, the binding of ligands to the receptor induces changes in the expression level of mRNAs encoded by PPAR target genes.

On the other hand, the mechanisms underlying transrepression and transactivation are very different. Transrepression is the independent regulation of gene expression that occurs *via* PPRE binding. PPARs can physically interact with other types of transcription factors and can influence their functions without binding to the DNA. Through this mechanism, PPARs suppress the activities of several transcription factors, including nuclear factor κB (NFκB), activation protein 1 (AP-1), signal transducers and activators of transcription (STAT), and the nuclear factor of activated T cells (NFAT) signaling pathways [23,24], *via* direct protein-protein interactions between PPAR and these transcription factors [25]. Transrepression may also result from competition for a limited supply of transcriptional coactivators or possibly by altered interactions with the basal transcription machinery. This transrepression activity probably constitutes the mechanical basis for the anti-inflammatory properties of PPARs.

IMMUNOLOGICAL EFFECTS OF PPARΓ AGONISTS AND EAE

PPARγ was originally characterized as a regulator of adipocyte differentiation and lipid metabolism [26-28]. There are two splice isoforms of PPARγ: PPARγ1and PPARγ2, which are both products of differential promoter usage and splicing. PPARγ2 has 28 additional N-terminal amino acids that confer a 5- to 6-fold increase in the transcription-stimulating activity of the ligand-independent activation function-1 domain. PPARγ1 and PPARγ2 showed similar tran-

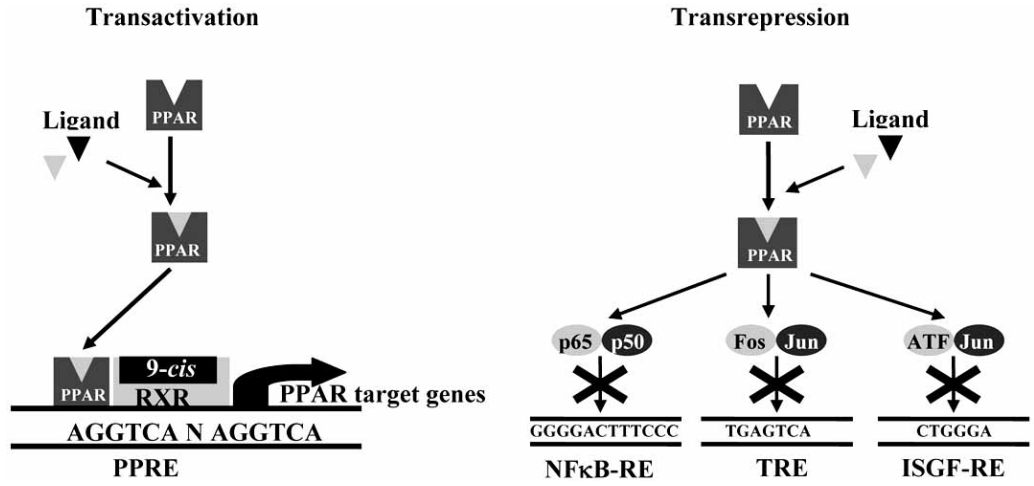

Fig. (2). Transactivation and Transrepression with PPARs. In transactivation, PPAR-RXR heterodimers bind to DNA-specific sequences of PPREs that are located in the promoter regions of the target genes. In transrepression, PPARs repress gene transcription by negatively interfering with the NFκB, AP-1 (Fos/Jun), and STAT signaling pathways in a DNA-binding independent manner. TRE: TPA-response element; ISGF-RE: interferon stimulated gene factor response element.

scriptional responses to PPAR agonists [10]. It was found that PPARγ is expressed in many kinds of immune or immune-associated cells. PPARγ is expressed on lymphoid organs [29] or in hematolymphoid lineage cells such as monocytes, bone-marrow precursors [30], macrophages [31], and microglias [32]. The receptor is also expressed in helper T cell clones [33] and B lymphocytes [34] as well as in neuronal cells [35]. It has been reported that PPARγ1 is expressed in the liver, adipocytes, macrophages, T cells and a few other cell types, whereas PPARγ2 is expressed exclusively in adipose tissues [11,36].

Recently, it has been suggested that several synthetic ligands for PPARγ, such as the anti-diabetic thiazolidinediones, e.g., pioglitazone and ciglitazone, function as important immunomodulatory factors.

T cells that are autoreactive to myelin antigens are likely to contribute to the initiation of MS, and there are several reports on the immunological effects of PPARγ agonists to T cells. The synthetic PPARγ ligands troglitazone and ciglitazone as well as the natural ligand 15d-PGJ_2 inhibited IL-2 production and phytohemagglutinin (PHA)-inducible proliferation in human peripheral blood T cells [37]. It was also reported that the PPARγ agonists suppressed the proliferative responses of both T cell clones and the freshly isolated splenocytes [33]. Inhibition in these cases is mediated directly at the T cell level and not at the macrophage/APC level. PPARγ ligands can also affect T cell function indirectly by inhibiting endothelial cell production of chemokines [38].

In recent years, there has been increasing interest regarding the potential of B cells and their contributions to MS. Interestingly, it was revealed that B cells express PPARγ, and the PPARγ ligands 15d-PGJ_2 and thiazolidinediones inhibit B cell proliferation and induce apoptosis of B cells [39,40].

Macrophages and monocytes also play a crucial role in the pathogenesis of MS. It was demonstrated that PPARγ is markedly up-regulated in murine-activated macrophages and that natural and synthetic PPARγ ligands inhibit the induction of inducible nitric oxide synthase (iNOS), matrix metalloproteinase-9 (MMP-9), and scavenger receptor A gene transcription [23]. PPARγ ligands can also induce the apoptosis of macrophages [41]. Furthermore, it was revealed that incubation of human monocytes with 15d-PGJ_2 or with synthetic agonists inhibits the production of proinflammatory cytokines, including TNFα, IL-1β, and IL-6, in part by antagonizing the activities of transcription factors such as AP-1 and NFκB [42].

Microglias are resident cells of the CNS that share similarities with peripheral macrophages. Microglias serve as antigen-presenting cells and are phagocytic. Additionally, when activated, they produce pro-inflammatory cytokines and chemokines, which play a critical role in the pathogenesis of MS (review [43]). Petrova *et al*. were the first to report that 15d-PGJ_2 potently inhibited LPS-induced iNOS and subsequent NO production in a dose-dependent manner in the BV-2 mouse microglial cell line [44]. 15d-PGJ_2 decreases the production of TNFα, IL-1β, and cyclooxygenase-2 (COX-2) in LPS-stimulated BV-2 microglial cells, thereby acting as a general inhibitor of microglial activation [45]. Similarly, using primary rat microglias, it was demonstrated that 15d-PGJ_2 suppressed TNFα, NO, and MHC-class II expression [32]. Drew and Chavis showed that 15d-PGJ_2 repressed the production of IL-12 as well as NO in both the mouse N9 microglial cell line and the microglia [46]. Recently, it was also shown that three types of thiazolidinediones rosiglitazone, pioglitazone, and ciglitazone are effective in inhibiting the production of NO; the pro-inflammatory cytokines TNFα, IL-1β, and IL-6; and the chemokine MCP-1 from microglias and astrocytes [47].

In MS, the sites of new or active brain lesions are enriched in activated lymphocytes. Accumulation of these cells involves enhanced transmigration across the blood brain barrier, proliferation within the brain, and possibly the enhanced survival of lymphocytes within the brain. As described previously, PPARγ interferes with chemo-attraction and cell adhesion of monocytes and lymphocytes in the vascular wall. PPARγ agonists have also been shown to selectively modulate the expression of various adhesion molecules such as MMP-9, monocyte chemotactic protein-1 (MCP-1), RANTES (CCL5), and vascular cell adhesion molecule-1 (VCAM-1) [23,48-52].

As described previously, 15d-PGJ_2 a natural ligand for PPARγ has several immunological effects. However, 15d-PGJ_2 inhibits the expression of a variety of immune response genes through PPARγ-independent mechanisms; these include 15d-PGJ_2 inhibition of NFκB activity through two distinct mechanisms. First, 15d-PGJ_2 inhibits IKK activation in response to inflammatory stimuli, blocking IκB degradation and thus preventing the nuclear translocation of NFκB [53,54]. In addition, 15d-PGJ_2 acts in a PPARγ-independent manner to directly inhibit NFκB binding to NFκB DNA-response elements. In this case, 15d-PGJ_2 acts by alkylating NFκB-rel proteins at specific cysteine residues, resulting in the inhibition of DNA binding [55]. It was also indicated that the cyclopentenone ring structure alone was capable of mediating inhibition of NFκB activity, thereby indicating that 15d-PGJ_2 possibly affects NFκB activity in a PPARγ-independent fasion [56]. However, interestingly, 15d-PGJ_2 the PPARγ agonist that shows the weakest binding to the receptor was the most potent in suppressing pro-inflammatory activity and NO production from both microglias and astrocytes [47].

Based on the immunomodulative data of PPARγ agonists, it was inferred that these agonists would have effects on several autoimmune diseases. It was shown that troglitazone, a synthetic PPARγ ligand, markedly reduced the development of disease in some of the autoimmune disease models, including those for IBD [13] and rheumatoid arthritis [14]. We previously demonstrated that troglitazone ameliorated EAE [57] (Table **1**). The clinical characteristics of EAE are focal areas of inflammation and demyelination and of an infiltrate containing a large number of lymphocytes and macrophages throughout the CNS. There are several combinations of mouse/rat strains and peptides for studying EAE. We used the myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 to C57BL/ 6 mice for inducing EAE; subsequently, troglitazone treatment was performed, and the drug-attenuated development of active EAE was demonstrated [57]. Enhanced mRNA expression of the PPARγ1 isoform

Table 1. Effects of PPAR Agonists to EAE

PPAR Subtype	PPAR Agonists	Administration of PPAR Agonists		Mouse Strain in the EAE Model	Peptide for EAE	Effects (Treatment Before Onset)	Effects (Treatment After Onset)	Reference
PPARγ	Troglitazone	50 or 100 mg/kg/day	gavage	C57BL/6	MOG35-55	●	○	[57]
	15d-PGJ_2	100 μg/kg/day to 1 mg/kg/day	i.p.	B10.PL	MBP Ac1-11	●	○	[58]
	Pioglitazone	120 ppm	mixed with chow	C57BL/6	MOG35-55	●	○	[59]
	Pioglitazone	120 ppm	mixed with chow	B10.PL	guinea pig MBP	▲	N/E	[59]
	Rosiglitazone	100 ppm	mixed with chow	C57BL/6	MOG35-55	●	N/E	[59]
	GW347845	100 ppm	mixed with chow	C57BL/6	MOG35-55	●	N/E	[59]
	15d-PGJ_2	50 or 100 μg/e.o.d.	i.p.	SJL/J	MSCH	●	N/E	[60]
	Ciglitazone	50 or 100 μg/e.o.d.	i.p.	SJL/J	MSCH	●	N/E	[60]
PPARα	Gemfibrozil	500 μg/day	gavage	B10.PL	MBP Ac1-11	●	○	[76]
	Gemfibrozil	0.25% w/w	mixed with chow	B10.PL	MBP Ac1-11	●	N/E	[76]
	Fenofibrate	500 μg/day	gavage	B10.PL	MBP Ac1-11	●	○	[76]
	Fenofibrate	0.25% w/w	mixed with chow	B10.PL	MBP Ac1-11	●	N/E	[76]
PPARδ/β	GW0742	100ppm	mixed with chow	C57BL/6	MOG35-55	●	○	[81]

e.o.d., every other day; i.p., intraperitoneal injection; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; MSCH, mouse spinal cord homogenate; ●, reduced maximum clinical score and/or delayed disease onset; ▲, no reduced clinical score but showed complete recovery; ○, reduced clinical score; N/E, not examined

but not of the PPARγ2 isoform was detected in the spinal cord of the troglitazone-treated mice when compared with the control mice; in the latter troglitazone appeared to increase PPARγ1 transcription of macrophages in the lesion [57]. Troglitazone treatment appeared to result in enhanced expression of the PPARγ mRNA and reduced the mRNA expression of the pro-inflammatory cytokines IL-1β and TNFα in the spinal cords of the mice. However, the controls and the troglitazone-treated mice did not show significant differences in IFNγ production from T cells on stimulation with the MOG peptide 35-55 [57]. Some reports stated that other PPARγ agonists ameliorated EAE (Table **1**). Diab *et al*. reported that 15d-PGJ_2 suppressed the development of EAE induced by the administration of MBP Ac_{1-11} in B10.PL mice, even when administered after the observation of clinical symptoms [58]. They also reported that 15d-PGJ_2 inhibited the proliferation of Ag-specific T cells from the spleen of MBP Ac_{1-11} TCR-transgenic mice and suppressed IFNγ, IL-10, and IL-4 production by both Con A- and MBP Ac_{1-11} peptide-stimulated lymphocytes [58]. Examination of the CNS during the course of EAE revealed the expression of PPARγ in the spinal cord inflammatory infiltrate [58]. Feinstein *et al*. also demonstrated that the oral administration of another PPARγ agonist pioglitazone reduced the incidence and severity of two EAE models: monophasic, chronic EAE in C57BL6 mice induced by the MOG peptide 35-55 and relapsing EAE in B10.PL mice induced by the guinea pig myelin basic protein (MBP). However, the disease onset was not delayed in monophasic EAE [59]. Using the relapsing EAE model, they also demonstrated that pioglitazone had no effect on the onset or severity of the initial disease attack: however, it reduced the severity of subsequent relapses and resulted in an overall decrease in mortality. They also demonstrated that other PPARγ agonists, namely, rosiglitazone and GW347845, also reduced the development of clinical signs of MOG-induced EAE [59]. Interestingly, although pioglitazone has the lowest affinity for PPARγ, it was the most effective among the agonists in reducing EAE. Pioglitazone reduced MIP1α, RANTES, and iNOS expression and increased IκBβ expression in the CNS of EAE [59]. Pioglitazone also reduced antigen-dependent IFNγ production from EAE-derived T cells [59]. Neural antigen-specific T cells

obtained from the spleen of myelin basic protein Ac_{1-11} TCR-transgenic mice can induce EAE on activation with an antigen; using these cells, it was shown that 15d-PGJ_2 inhibited T cell proliferation [58]. Additionally, this agonist suppressed IFNγ, IL-10, and IL-4 production by activated lymphocytes [58]. It also suppressed the development of EAE, even when administered after the clinical symptoms were observed [58]. It was demonstrated that ciglitazone, another PPARγ agonist, exerts protective effects in EAE of SLJ/J mice immunized by a mouse spinal cord homogenate (MSCH) [60]. They also reported that treatment of mice with ciglitazone or 15d-PGJ_2 decreased the clinical severity of adoptive transfer EAE [60]. Ciglitazone or 15d-PGJ_2 resulted in a dose-dependent decrease in the proliferation of MBP-immune T cells and IFNγ production from MBP-immune spleen cells as well as inhibition of IL-12 production from macrophage and microglial cells [60]. Recently, it was also reported that the PPARγ antagonists reverse the inhibition of EAE by the PPARγ agonists, ciglitazone and 15d-PGJ_2, in C57BL/6 wild-type mice and PPARγ-deficient heterozygous mice [61]. The reversal of EAE by PPARγ antagonists was associated with the restoration of neural antigen-induced T cell proliferation, IFNγ production and Th1 differentiation that were inhibited by ciglitazone and 15d-PGJ_2 [61]. These results suggest that ciglitazone and 15d-PGJ_2 ameliorate EAE through PPARγ-dependent mechanisms.

As described previously, PPARs regulate gene expression by binding as heterodimers with RXRs to specific PPREs in the promoter regions of specific target genes. It was reported that administration of the RXR ligand 9-*cis*-retinoic acid (RA) alone at the onset of clinical signs of EAE reduced the severity of the disease, and a combination of RA and the PPARγ ligand 15d-PGJ_2 resulted in enhanced amelioration of the disease, which might be attributable to the inhibition of T cell proliferation and cytokine production of IFNγ [62]. The additive anti-inflammatory effect of RXR and PPARγ agonists could be beneficial in a clinical setting because it might prevent adverse events that are often encountered when these agonists are used in monotherapy at considerably high doses.

Recently, it was reported that the PPARγ-deficient heterozygous mice exacerbated EAE with prolonged clinical symptoms than the wild-type mice, and the exacerbation of EAE in PPARγ deficient heterozygous mice following immunization with MOG peptide 35-55 was associated with a significant increase in $CD4^+$, $CD8^+$, MHC class II^+, and $CD40^+$ cells [63]. PPARγ-deficient heterozygous mice also showed a significant increase in neural Ag-induced T cell proliferation, IL-12/IFNγ secretion, and Th1 differentiation than their wild-type littermates [63]. It was also shown that the C57BL/6 wild-type mice develop an exacerbated EAE with prolonged clinical symptoms following treatment with PPARγ antagonists, which was comparable to the disease profile in PPARγ-deficient heterozygous mice [64]. The exacerbation of EAE by PPARγ antagonists was associated with augmented neural antigen-induced T cell proliferation, IFNγ production, or Th1 differentiation [64]. These results support the idea that PPARγ is a critical physiological regulator of CNS inflammation and demyelination in EAE.

IMMUNOLOGICAL EFFECTS OF PPARA AGONISTS AND EAE

PPARα agonists have also been shown to regulate inflammatory responses, although evidence for this is considerably less as compared with that for PPARγ agonists. PPARα-deficient mice have abnormally prolonged responses to inflammatory stimuli such as arachidonic acid and leukotrienes [65]. PPARα expression is noted in lymphocytes; it is higher in B cells than in T cells; and its expression wanes shortly after lymphocyte activation [66]. PPARα is also expressed in human endothelial cells (ECs) [67] and in human monocytes [68]. PPARα agonists have been shown to transcriptionally repress (transrepress) the expression of vascular cell adhesion molecules in human vascular ECs [67]. These agonists also repress cytokine-induced IL-6 production in smooth muscle cells [24] and inducible nitric-oxide synthase activity in murine macrophages [69]. The PPARα agonist WY14643 was shown to decrease NFκB activation and the production of IL-12 and IL-6 in aged mice [70] and also inhibit IL-2, TNFα, and IFNγ production by activated $CD4^+$ T cells [71]. The agonist also markedly inhibited IFNγ expression and markedly augmented IL-4 expression in splenocytes [72]. On the other hand, treatment with a potent and specific PPARα ligand, namely, GW7647, did not augment IL-4. Additionaly, WY14643 induced IL-4 expression in splenocytes from PPARα knockout mice, suggesting that the effect of WY14643 on IL-4 was largely *via* a PPARα-independent mechanism [73]. It was demonstrated that WY14643 caused splenocyte depletion and induced apoptosis in both B and T cells [72] and PPARα agonists have been shown to induce apoptosis in macrophages [68]. The PPARα agonists fenofibrate and WY14643 inhibited NO production from microglias and astrocytes [74,75]. These agonists also inhibited the production of the pro-inflammatory cytokines IL-1β and TNFα, which are potentially toxic to cells, including the myelin-producing oligodendrocytes from the microglias. In addition, these agonists inhibit the microglial production of IL-12 p40 and MCP-1 [74]. Furthermore, a combination of PPARα and RXR agonists jointly inhibited NO production as well as the production of pro-inflammatory cytokines, including IL-1β, TNFα, and IL-6, from the microglias and astrocytes [73,75]. The PPARα agonists gemfibrozil and fenofibrate suppressed proliferation by TCR transgenic T cells specific for the myelin basic protein Ac_{1-11} as well as reduced NO production from the microglias [76]. Interestingly, gemfibrozil was shown to shift cytokine secretion in human T-cell lines by inhibiting IFNγ and by promoting IL-4 secretion [76].

Considering the immunomodulatory effects of WY14643, Cunard *et al*. attempted to treat EAE with the drug; however, they were unsuccessful because a combination of MOG peptide 35-55/complete Freund's adjuvant, the pertussis toxin, and WY14643 administration consistently leads to a very high mortality rate after 5-10 days of immunization, the reason for which remains unclear [72]. However, it was recently reported that the administration of other agonists for PPARα could be attempted for EAE treatment without resulting in mortality (Table **1**). Oral administration of gemfibrozil and fenofibrate inhibited the development of the clinical signs of EAE [76]. Furthermore, gemfibrozil was shown to shift the

cytokine secretion of human T cell lines by inhibiting IFNγ and promoting IL-4 secretion [76].

IMMUNOLOGICAL EFFECTS OF PPARΔ/B AGONISTS AND EAE

Although PPARδ/β is expressed in many neural cell types [77,78], it was shown to be the major PPAR isotype expressed in optic nerve oligodendrocytes [79] and sciatic nerve Schwann cells. In primary cultures from neonatal mice, PPARδ/β was found to be highly expressed in oligodendrocytes but not in astrocytes [79]. PPARδ/β showed the highest expression in immature oligodendrocytes, suggesting its role in oligodendrocyte development. In fact, the treatment of cultures containing immature oligodendrocytes with selective PPARδ/β agonists showed increased oligodendrocyte maturation, although without strong effects on oligodendrocyte proliferation [80]. It was also shown that PPARδ/β-selective agonists induced oligodendrocyte differentiation and increased expression of MBP and proteolipid protein (PLP) mRNA [80]. Recently, it was revealed that oral administration of the selective PPARδ/β agonist GW0742 reduced clinical symptoms in EAE in C57BL/6 mice that had been immunized with the encephalitogenic MOG peptide (Table **1**). GW0742 showed moderate attenuation in the clinical symptoms when provided simultaneously with immunization, and this result is different from the effects of PPARγ agonists seen thus far. However, a greater reduction was observed when this drug was administered during disease progression. One of the mechanisms suggested by the authors was that GW0742 reduced astroglial and microglial inflammatory activation and IL-1β levels in the brain in EAE, and it increased the expression of some myelin genes [81]. These data showed that PPARδ/β agonists, similar to other PPAR ligands, can exert protective actions in EAE and can be possibly applied to MS.

PPARs AND MS

Recently, it was reported that pioglitazone and ciglitazone inhibit proliferative responses and the secretion of the proinflammatory cytokine of TNFα and IFNγ from PHA-stimulated human PBMCs derived from healthy controls and MS patients and human T leukemia cells (Jurkat cells) [82, 83]. These effects are much more pronounced when the cells are preincubated with pioglitazone prior to inflammatory stimulation, indicating a sensitizing effect induced by pioglitazone pretreatment [83]. In patients with MS, these anti-inflammatory effects of pioglitazone treatment were significantly reduced as compared to those in healthy controls [83]. Interestingly, untreated PBMCs from MS patients exhibited a strong reduction in PPARγ expression, and inflammatory stimulation of PBMCs from healthy controls resulted in an equal loss of PPARγ expression, suggesting that inflammatory conditions affect PPARγ expression. Differences in PPARγ expression were accompanied by changes in the PPARγ DNA-binding activity. In MS patients, the pioglitazone-induced increase in the PPARγ DNA-binding activity and a concomitant decrease in the NFκB DNA-binding activity were only observed in the absence of an acute inflammatory event [83]. These results suggest that treatment with pioglitazone can prevent the inflammation-induced loss of PPARγ activity only when administered prior to an acute inflammatory event. With regard to the therapeutic potential of PPARγ agonists in the treatment of MS, these observations underline the significance of early onset of treatment before an acute relapse.

The thiazolidinediones represent a class of drugs that are insulin sensitizing and are a major therapeutic advancement in the treatment of type 2 diabetes. We initially reported their effects in EAE by using troglitazone as a representative PPARγ agonist, which was the first thiazolidinediones to reach the market. Unfortunately, this drug was completely withdrawn from clinical use in 2000 due to reports of severe, idiosyncratic hepatotoxicity and its use in the treatment of MS is therefore discontinued. Today, two thiazolidinediones pioglitazone and rosiglitazone are commercially available antidiabetic agents having similar clinical efficacy in improving insulin sensitivity and have the ability to lower fasting blood glucose levels. Recently, a patient with secondary progressive MS treated with daily oral administration of pioglitazone for 3 years showed apparent clinical improvement without adverse events [84]. Clinical trials of PPARγ agonists are in progress for MS and Alzheimer disease.

CONCLUSIONS

As described above, certain PPAR agonists have been revealed to have immunosuppressive effects on several immune cells such as macrophages, lymphocytes, and microglias and are known to suppress the development of EAE. However, further studies are needed to clarify the role of PPARs in EAE and to determine whether PPAR agonists, which are presently used for treatment in humans, can also present as a useful therapeutic target to MS. The answers to these questions will direct future efforts in the prevention and treatment of MS.

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