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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

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Abstract

Peroxisome proliferator-activated receptor-gamma is a nuclear receptor transcription factor that regulates cell growth, differentiation and homeostasis. PPARγ agonists have been used to treat obesity, diabetes, cancer and inflammation and recent studies have shown the protective effects of PPARγ agonists on experimental allergic encephalomyelitis (EAE), a Th_1 cell-mediated autoimmune disease model of multiple sclerosis (MS). Our studies have further demonstrated that the PPARγ agonists, 15d-PGJ_2 and Ciglitazone, inhibit EAE through blocking IL-12 signaling leading to Th_1 differentiation and the PPARγ deficient heterozygous mice (PPARγ$^{+/-}$) or those treated with PPARγ antagonists develop an exacerbated EAE in association with an augmented Th_1 response. In this study, we show that the PPARγ antagonists, Bisphenol A diglycidyl ether (BADGE) and 2-chloro-5-nitro-*N*-(4-pyridyl)benzamide (T0070907), reverse the inhibition of EAE by the PPARγ agonists, Ciglitazone and 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2, in C57BL/6 wild-type and PPARγ$^{+/-}$ mice. The reversal of EAE by BADGE and T0070907 was associated with restoration of neural antigen-induced T cell proliferation, IFNγ production and Th_1 differentiation inhibited by Ciglitazone and 15d-PGJ_2. These results suggest that Ciglitazone and 15d-PGJ_2 ameliorate EAE through PPARγ-dependent mechanisms and further confirm a physiological role for PPARγ in the regulation of CNS inflammation and demyelination in EAE.

Keywords: EAE/MS; PPARγ; Th_1 response; Inflammation; Autoimmunity; Demyelination

1. Introduction

Multiple sclerosis (MS) is a neurological disorder that affects more than 2.5 million people worldwide (Noseworthy et al., 2000; Bitsch and Bruck, 2002). The disease usually begins in young adulthood and affects women more frequently than men (Wingerchuk et al., 2001). About 30% of MS patients develop clinical paralysis and become wheel chair-bound for rest of their lives (Bitsch and Bruck, 2002). While destruction of oligodendrocyte myelin sheath and manifestation of focal sclerotic lesions in the CNS are the pathological hallmark of MS, axonal degeneration contributes to irreversible long-term disability (Steinman et al., 2002; Franklin, 2002; Coleman and Perry, 2002). Although the etiology of MS is not known, it is generally viewed as an organ-specific autoimmune disease, mediated by myelin-reactive T cells in the CNS (Steinman et al., 2002; Hemmer et al., 2002). Activation of immune cells, secretion of inflammatory cytokines and differentiation of encephalitogenic Th_1 cells are key processes associated with the pathogenesis of MS (Noseworthy et al., 2000; Wingerchuk

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et al., 2001; Steinman et al., 2002). Experimental allergic encephalomyelitis (EAE) is a $CD4^+$ Th_1 cell-mediated inflammatory demyelinating autoimmune disease of the CNS (Gold et al., 2000; Owens and Sriram, 1995). EAE can be induced in susceptible strains of rodents and primates by immunization with neural-antigens such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP) or by adoptive transfer of neural antigen-sensitized T cells. The clinical and pathological features of EAE show close similarity to human MS and therefore has been commonly used as a model system to study the mechanism of MS pathogenesis and to test the efficacy of potential therapeutic agents for the treatment of MS (Gold et al., 2000; Owens and Sriram, 1995; Bright et al., 1998, 1999).

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the subfamily of ligand-dependent nuclear receptor transcription factors that regulates lipid metabolism, glucose homeostasis, tumor progression and inflammation (Evans, 1988; Blumberg and Evans, 1998; Mukherjee et al., 1997; Elbrecht et al., 1996). PPARγ is expressed predominantly in adipose tissue, heart, kidney, pancreas, spleen, intestine, colon epithelial cells and skeletal muscle and the PPARγ mutant homozygous mice die by day E10 due to developmental defects (Barak et al., 1999). Several fatty acids and ecosanoids including 9-hydroxyoctadecadienoic acid (HODE) and 13-HODE function as physiological ligands for PPARγ (Willson and Wahli, 1997; Krey et al., 1997). The 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 (15d-PGJ_2) is a high affinity natural ligand that activates PPARγ at nanomolar concentrations (Forman et al., 1995). The thiazolidinediones (TZD) class of compounds such as rosiglitazone, pioglitazone, troglitazone, and ciglitazone are high affinity synthetic agonists for PPARγ (Lehmann et al., 1995). PPARγ agonists regulate adipogenesis and prevent obesity (Kliewer and Willson, 1998). PPARγ agonists also modulate glucose metabolism and insulin sensitivity, thereby reducing plasma glucose and insulin levels in type 2 diabetes (Schwartz et al., 1998; Barroso et al., 1999). PPARγ agonists inhibit malignant growth of different tumor cells (Demetri et al., 1999; Elstner et al., 1998), suggesting their use in the treatment of cancer. Recent studies have also demonstrated the anti-inflammatory effects of pharmacological agents that activate PPARγ in vitro and in vivo. For example, in vitro treatment with PPARγ ligands, 15d-PGJ_2 and rosiglitazone, repressed the expression of several inflammatory response genes in activated macrophages, including inducible nitric oxide synthase (iNOS), TNFα, IL-6, IL-1, gelatinase B, and cyclooxygenase 2 (COX-2) (Ricote et al., 1998; Jiang et al., 1998). In vivo treatment with PPARγ agonists attenuate the inflammatory diseases such as experimental colitis, adjuvant-induced arthritis, atherosclerosis, experimental myocarditis and sepsis in mice and rats (Kawahito et al., 2000; Neve et al., 2000).

We and others have shown recently that in vivo treatment with PPARγ agonists inhibit CNS inflammation and demyelination in EAE (Natarajan and Bright, 2002; Niino et al., 2001; Diab et al., 2002; Feinstein et al., 2002; Schmidt et al., 2004). Our findings also demonstrated that PPARγ agonists, 15d-PGJ_2 and Ciglitazone, ameliorate EAE by blocking IL-12 signaling through JAK–STAT pathway leading to Th_1 differentiation (Natarajan and Bright, 2002). Further analyses showed that the PPARγ deficient heterozygous mice develop an exacerbated EAE in association with augmented neural antigen-specific Th_1 response, suggesting a physiological role for PPARγ in the regulation of inflammation and demyelination in EAE (Natarajan et al., 2003). Interestingly, recent studies have also identified synthetic compounds including, bisphenol A diglycidyl ether (BADGE) and 2-chloro-5-nitro-*N*-(4 pyridyl)benzamide (T0070907) as PPARγ antagonists which bind to PPARγ but has no ability to induce transcriptional activity of PPARγ, rather antagonize PPARγ agonists to activate transcriptional and adipogenic actions of this receptor (Wright et al., 2000). We have demonstrated recently that in vivo treatment with the PPARγ antagonists, BADGE and T007, exacerbates EAE in association with augmented neural antigen-specific Th_1 response (Raikwar et al., 2005). While, the PPARγ antagonist GW9662 reverse the actions of synthetic PPARγ agonists in tumor cells (Betz et al., 2005), 15d-PGJ_2 regulates lipid metabolism and inflammation through both PPARγ independent and dependent mechanisms (Chawla et al., 2001; Giri et al., 2004). In this study, we show that in vivo treatment with these PPARγ antagonists reverse the inhibition of Th_1 response and EAE by 15d-PGJ_2 and Ciglitazone in wild-type and PPARγ deficient heterozygous mice, suggesting a critical physiological role for PPARγ in the regulation of CNS inflammation and demyelination.

2. Materials and methods

2.1. Animals

The C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, Maine). The PPARγ deficient heterozygous mice ($PPAR\gamma^{+/-}$) were generated as described earlier (Barak et al., 1999) and maintained in the animal care facility at Vanderbilt University Medical Center (Raikwar et al., 2005). 4 to 6 weeks old female mice were used in the experiments. All the animal protocols used in the experiments were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

2.2. Reagents

The PPARγ antagonist, bisphenol A diglycidyl ether (BADGE, $C_{21}H_{24}O_4$) was purchased from Fluka Chemicals (St Louis, MO) and 2-chloro-5-nitro-*N*-(4 pyridyl)benzamide (T0070907, $C_{12}H_8ClN_3O_3$) was purchased from Calbiochem (La Jolla, CA). The PPARγ agonist, 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2, was purchased from