ASN NEURO RESEARCH ARTICLE

ASN NEURO 2(1):art:e00025.doi:10.1042/AN20090033

Regulation of oligodendrocyte progenitor cell maturation by PPARδ: effects on bone morphogenetic proteins

Maria Vittoria Simonini*, Paul E Polak*,†, Anne I Boullerne*,†, Jeffrey M Peters‡, Jill C Richardson and Douglas L Feinstein*,‡[1]

*Department of Anesthesiology, University of Illinois, Chicago, IL 60612, U.S.A.
†Department of Veterans' Affairs, Jesse Brown VA, Chicago, IL 60612, U.S.A.
‡Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802, U.S.A.
GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Harlow, Essex CM19 5AW, U.K.

Cite this article as: Simonini MV, Polak PE, Boullerne AI, Peters JM, Richardson JC and Feinstein DL (2010) Regulation of oligodendrocyte progenitor cell maturation by PPARδ: effects on bone morphogenetic proteins. ASN NEURO 2(1):art:e00025.doi:10.1042/AN20090033

ABSTRACT

In EAE (experimental autoimmune encephalomyelitis), agonists of PPARs (peroxisome proliferator-activated receptors) provide clinical benefit and reduce damage. In contrast with PPARγ, agonists of PPARδ are more effective when given at later stages of EAE and increase myelin gene expression, suggesting effects on OL (oligodendrocyte) maturation. In the present study we examined effects of the PPARδ agonist GW0742 on OPCs (OL progenitor cells), and tested whether the effects involve modulation of BMPs (bone morphogenetic proteins). We show that effects of GW0742 are mediated through PPARδ since no amelioration of EAE clinical scores was observed in PPARδ-null mice. In OPCs derived from E13 mice (where E is embryonic day), GW0742, but not the PPARγ agonist pioglitazone, increased the number of myelin-producing OLs. This was due to activation of PPARδ since process formation was reduced in PPARδ-null compared with wild-type OPCs. In both OPCs and enriched astrocyte cultures, GW0742 increased noggin protein expression; however, noggin mRNA was only increased in astrocytes. In contrast, GW0742 reduced BMP2 and BMP4 mRNA levels in OPCs, with lesser effects in astrocytes. These findings demonstrate that PPARδ plays a role in OPC maturation, mediated, in part, by regulation of BMP and BMP antagonists.

Key words: astrocyte, bone morphogenetic protein (BMP), experimental autoimmune encephalomyelitis (EAE), multiple sclerosis, myelin, noggin.

INTRODUCTION

Studies from our laboratory (Feinstein et al., 2002) and others (Diab et al., 2002; Natarajan and Bright, 2002) have shown that agonists of PPARγ (peroxisome proliferator-activated receptor γ) reduce clinical and histological symptoms in EAE (experimental autoimmune encephalomyelitis), an animal model of MS (multiple sclerosis). These effects are due, in part, to suppression of inflammatory gene expression (Drew et al., 2006; Xu et al., 2007), inhibition of activated T-cell proliferation and production of inflammatory mediators (Feinstein, 2003; Kielian and Drew, 2003), and reduction of leucocyte infiltration into the CNS (central nervous system) (Klotz et al., 2007). These findings have led to the design of small clinical trials in relapsing remitting MS patients (Miller et al., 2005; Kaiser et al., 2009) with encouraging results. Similarly, agonists of PPARα show anti-inflammatory actions on glial cells (Deplanque et al., 2003; Xu et al., 2005, 2006) and benefit in EAE (Lovett-Racke et al., 2004; Dasgupta et al., 2007; Xu et al., 2007).

In contrast with PPARα and PPARγ, studies of the PPARδ (also referred to as PPARβ or PPARβ/δ) isoform in EAE are

[1]To whom correspondence should be addressed (email dlfeins@uic.edu).

Abbreviations: *ANGPTL-4*, angiopoietin-like 4; bFGF, basic fibroblast growth factor; bHLH, basic helix-loop-helix; BMP, bone morphogenetic protein; *CGT*, galactose ceramide galactosyl transferase; CNS, central nervous system; DMEM, Dulbecco's modified Eagle's medium; E13 etc., embryonic day 13 etc; EAE, experimental autoimmune encephalomyelitis; *GalC*, galactosyl ceramidase; GDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; Id, inhibitors of differentiation; IFNγ, interferon γ; MBP, myelin basic protein; MOG_{35-55}, myelin oligodendrocyte glycoprotein peptide 35–55; MS, multiple sclerosis; NPM, neural proliferation medium; ODM, oligodendrocyte differentiation medium; OL, oligodendrocyte; Olig, OL transcription factor; OPC, OL progenitor cell; P1 etc., post-natal day 1 etc; PDGFα, platelet-derived growth factor α; PDL, poly-D-lysine; PLP, proteolipid protein; PPAR, peroxisome proliferator-activated receptor; PT, pertussis toxin; qPCR, quantitative PCR; TNFα, tumour necrosis factor α; TRITC, tetramethylrhodamine β-isothiocyanate; UCP, uncoupling protein; WT, wild-type.

limited. PPARδ is expressed throughout the body in most tissues and is the most abundant PPAR in brain (Michalik et al., 2003). The knowledge that activation of PPARδ regulates lipid metabolism (Basu-Modak et al., 1999; Kliewer et al., 2001; Rosenberger et al., 2002), raised the possibility that PPARs might regulate lipid metabolism in OLs (oligodendrocytes). Studies from the Skoff laboratory (Granneman et al., 1998) have shown that PPARδ is the major PPAR isotype expressed in optic and sciatic nerve, and is mainly expressed in the OL population. A subsequent study (Saluja et al., 2001) confirmed that PPARδ, but not PPARγ, selective agonists increased OL differentiation, including increasing MBP (myelin basic protein) and PLP (proteolipid protein) protein and mRNA levels.

Based on the above findings, we previously tested whether PPARδ agonists could provide protection in EAE (Polak et al., 2005). We found that, in contrast with PPARα and PPARγ agonists, treatment with a PPARδ agonist did not significantly reduce disease severity during the early stages of EAE, but instead showed benefit when given at the peak of disease. This was accompanied by reductions in the appearance of cortical lesions, neuronal damage and glial inflammation. Moreover, in contrast with PPARγ agonists, the selective PPARδ agonist GW0742 did not suppress pro-inflammatory cytokine production from T-cells, which may account for its reduced efficacy to influence early stages of EAE. GW0742 also caused an increase in myelin gene expression in EAE brains, suggesting a distinct mechanism of action possibly involving effects on OL maturation or survival.

Among the many factors implicated in OPC (OL progenitor cell) maturation are members of the BMP (bone morphogenetic protein) family. BMPs were originally identified as extracellular factors able to induce bone formation, but were later shown to be expressed in other tissues and play a role in development of other organs, including the nervous system (Goumans and Mummery, 2000; ten Dijke et al., 2003). BMPs belong to the TGF (transforming growth factor) superfamily which, upon binding to their cognate receptors, activate phosphorylation of Smad proteins, which in turn bind to specific promoter elements and regulate gene transcription. A key gene target of BMP signalling are Id (inhibitors of differentiation) proteins similar in structure to bHLH (basic helix-loop-helix) transcription factors, but lacking the DNA-binding domain (Miyazono and Miyazawa, 2002). Id proteins can therefore form heterodimers with other bHLH proteins, but the resulting complex is inactive. Both BMPs and their cognate receptors have been shown to be expressed by OLs during normal development (Cheng et al., 2007; See and Grinspan, 2009).

BMP signalling is regulated by interactions with a class of molecules referred to collectively as BMP antagonists (Yanagita, 2005) which function primarily by direct association with BMPs, thereby preventing binding to BMP receptors. BMP signalling has been shown to play a role in OL maturation and survival, since treatment with BMP antagonists promotes OL maturation (Mehler et al., 1997; Mabie et al., 1999; Mehler et al., 2000; Mekki-Dauriac et al., 2002), and the BMP antagonist noggin induced oligodendrogenesis in human embryonic stem cells (Izrael et al., 2007). The ability of BMPs to reduce OL maturation is due in large part to formation of complexes between Id proteins and the bHLH proteins Olig1 (OL transcription factor 1) and Olig2 which have been well characterized for their involvement in OL maturation (Cheng et al., 2007; Bilican et al., 2008). Given the importance of this signalling system in OL maturation, and in view of the fact that GW0742 increased myelin expression, we hypothesized that the effects of PPARδ agonists could involve modulation of the BMP signalling system.

In the present study we have used PPARδ-null mice to show that the beneficial effects of GW0742 in EAE are dependent upon the presence of functional PPARδ, and that PPARδ plays a role in regulating the normal processes of OL maturation. We also demonstrate that the effects of PPARδ involve regulation of BMP and BMP antagonist expression in OPCs and astrocytes. Taken together these findings support the concept that PPARδ plays an important role in the normal maturation of OPCs, and suggest that PPARδ agonists provide benefit in EAE by accelerating OPC maturation.

MATERIAL AND METHODS

Animals

Female C57BL/6 mice, aged 6–8 weeks, were from Charles River Breeding. PPARδ-null mice were generated as previously described (Peters et al., 2000). Pregnant Sprague–Dawley rats were purchased from Charles River Breeding, and used to provide P1 (where P is post-natal day) pups. Mice were maintained in a controlled 12 h:12 h light/dark environment and provided food *ad libitum*. All experiments were approved by the local IACUC (Institutional Animal Care and Use Committee).

Cell culture

Enriched cultures of primary mouse or rat astrocytes were prepared from P1 pups using procedures described previously, including complete change of media [DMEM (Dulbecco's modified Eagle's medium) containing 10% fetal calf serum and antibiotics] every 3 days (Dello Russo et al., 2003). The cells reached confluency after 7–8 days. At that time the cells were shaken for 2 h to remove adhering microglia, and overnight at 225 rev./min at 37°C to dislodge OPCs. The remaining cells are approximately 95% astrocytes by staining for the astrocyte-specific protein GFAP (glial fibrillary acidic protein), and 5% adherent microglia.

OPCs were prepared from E13 (where E is embryonic day) mouse pups using a recently described method (Pedraza et al., 2008). In brief, E13 embryos were removed, and then washed in cold PBS; the cerebellum was removed and meninges dissected away. The tissue from six to eight brains was triturated by 40 passages through a 1 ml pipette tip in DMEM/F12 and B27 neuronal supplement, and then filtered through a 70 μm-pore-size cell strainer. The cells were plated at the equivalent of two brains per T25 flask in 8 ml of NPM (neural proliferation medium) containing DMEM/F12/B27 neuronal supplement and 10 ng/ml EGF (epidermal growth factor; Sigma). The cells were passaged every 3 days by trituration and plated at a 1:5 ratio in NPM. After two passages, the cells were mechanically dissociated with a 1 ml pipette, then plated on to PDL (poly-D-lysine)-coated coverslips in NPM supplemented with 10 ng/ml bFGF (basic fibroblast growth factor) and 10 ng/ml PDGFα (platelet-derived growth factor α) [this is ODM (OL differentiation medium)].

For the data shown in Figure 2, cells were grown for 48 h on PDL-coated coverslips in ODM, the medium was then changed, and cells grown for a further 5 days in Sato–Bottenstein media in the absence of bFGF/PDGF, but containing 30 nM T3 and 10 μM AraC to reduce astrocyte proliferation, and either 10 μM of the PPARγ agonist pioglitazone, 10 μM of the PPARδ agonist GW0742, or the equivalent amount of DMSO vehicle to determine whether these drugs would induce further OPC maturation.

Induction of EAE

EAE was actively induced in 6–8-week-old mice using synthetic $MOG_{35–55}$ (myelin OL glycoprotein peptide 35–55) as described previously (Feinstein et al., 2002). The $MOG_{35–55}$ peptide (MEVGWYRSPFSRVVHLYRNGK) was purchased from Anaspec. Mice were injected subcutaneously (two 100 μl injections into adjacent areas in one hind limb) with an emulsion of 300 μg of $MOG_{35–55}$ dissolved in 100 μl of PBS, mixed with 100 μl of complete Freund's adjuvant containing 500 μg of *Mycobacterium tuberculosis* (Difco). Immediately after $MOG_{35–55}$ injection, the animals received an i.p. (intraperitoneal) injection of 200 ng of PT (pertussis toxin; List Biochemicals) in 200 μl of PBS. At 2 days later, the mice received a second PT injection, and 1 week later they received a booster injection of $MOG_{35–55}$.

Clinical assessment of EAE

Clinical signs were scored on a 5 point scale: grade 0, no clinical signs; 1, limp tail; 2, impaired righting; 3, paresis of one hind limb; 4, paresis of two hind limbs; 5, death. Scoring was performed at the same time each day by a blinded investigator.

Treatment of mice with PPARδ agonist

The selective PPARδ agonist GW0742 {4-[2-(3-fluoro-4-trifluoromethylphenyl)-4-methylthiazol-5-ylmethylsulfanyl]- 2-methylphenoxy)-acetic acid} was synthesized at GlaxoSmithKline as described previously (Sznaidman et al., 2003) and was provided by Dr Tim Willson (GlaxoSmithKline, Raleigh, NC, U.S.A.). Chow containing 100 p.p.m. GW0742 was prepared by Research Diets by mixing 100 mg of drug with 1 kg of Purina mouse chow 5001. Mice were provided free access to chow, and on average consumed 2 g per mouse per day, giving an average daily dose of 10 mg of GW0742/kg. This dose is similar to that previously use by our group and others for studies of other PPAR agonists in EAE models (Feinstein et al., 2002).

mRNA analysis

Total RNA from cells and tissues was isolated using TRIzol® reagent (Invitrogen/Gibco); aliquots were converted into cDNA using random hexamer primers, and mRNA levels estimated by qPCR (quantitative PCR). PCR conditions were 35 cycles of denaturation at 94°C for 10 s, annealing at 58–64°C for 15 s and extension at 72°C for 20 s on a Corbett Rotorgene real-time PCR unit. PCR was performed using Taq DNA polymerase (Invitrogen), and contained SYBR Green (SybrGreen1 10000× concentrate, diluted 1:10000; Molecular Probes). Relative mRNA concentrations were calculated from takeoff point of reactions using the software provided by the manufacturer, and normalized to α-tubulin and GDH (glyceraldehyde-3-phosphate dehydrogenase) measured in the same samples. Melting curve analysis and agarose gel electrophoresis ensured production of single and correct sized products. Primers were derived from published sequence information using Perl Primer software (perlprimer.sourceforge.net) to generate PCR products ranging from 100 to 250 bp. The primers used were: *GDH* forward, 5 -GCCAAGTATGATGACATCAAGAAG-3 ; *GDH* reverse, 5 -TCCAGGGGTTTCTTACTCCTTGGA-3 ; *Olig1* forward, 5 -ATGCCATCGGTGTTCGGACTTACT-3 ; *Olig1* reverse, 5 -TGTGGTTAAGGACCAGCCTGTGAA-3 ; *GalC* (galactosyl ceramidase) forward, 5 -AGGACATGCGGACGTTACAGCTAA-3 ; *GalC* reverse, 5 -TCCATAGGATCGTGCCGTTCAACA-3 ; *CGT* (galactose ceramide galactosyl transferase) forward, 5 -ACATTTGCAGTTCTCCTTGCTGCC-3 ; *CGT* reverse, 5 -AAGGCTACTGAGTTGGGCTGATGT-3 ; *PDGFR-α* forward, 5 -ACCTTGCACAATAACGGGAG-3 ; *PDGFR-α* reverse, 5 -GAAGCCTTTCTCGTGGACAG-3 ; *PPARδ* forward, 5 -GCCCAAGTTCGAGTTTGCTGTCAA-3 ; *PPARδ* reverse, 5 -TTAGCCACTGCATCATCTGGG-3 ; *UCP3* (uncoupling protein 3) forward, 5 -GAGAGGAAATACAGAGGGAC-3 ; *UCP3* reverse, 5 -GGGAGGTTGTCAGTAAACAG-3 ; *ANGPTL-4* (angiopoietin-like 4) forward, 5 -GCCACCCACTTACACAGGCCG-3 ; *ANGPTL-4* reverse, 5 -CCAGGCCCAGCCAGAACTCG-3 ; *Noggin* forward, 5 -TGAGCAAGAAGCTGAGGAGGAAGT-3 ; *Noggin* reverse, 5 -AGGTGCACAGACTTGGATGGCTTA-3 ; *Gremlin* forward, 5 -ACAGAGCGCAAGTATCTGAAGCGA-3 ; *Gremlin* reverse, 5 -AGGAGTTGCACTGGCCATAACAGA-3 ; *Follistatin* forward, 5 -TGGATCTTGCAACTCCATCTCGGA-3 ; *Follistatin* reverse, 5 -TGCCCAAAGGCTATGTCAACACTG-3 ; *Bambi* forward, 5 -TTCTGTGTTTGCCTGGCCTGTTTC-3 ; *Bambi* reverse, 5 -AGCAAGCTGTGGAGAGGTCAAGAR-3 ; *BMP2* forward, 5 -TGATCACCTGAACTCCACCAACCA-3 ; *BMP2* reverse, 5 -AACCCTCCACAACCATGTCCTGAT-3 ; *BMP4* forward, 5 -AGAACTGCCGTCGCCATTCACTAT-3 ; and *BMP4* reverse, 5 -AGTTGAGGTGATCAGCCAGTGGAA-3 .

Immunocytochemical staining

E13 OPCs were grown on PDL-coated coverslips, treated and prepared for staining. Coverslips were then rinsed in PBS, fixed with 4% PFA (paraformaldehyde) for 10 min, rinsed in PBS, incubated with mouse anti-MBP (1:200; Chemicon), goat anti-noggin (1:40; R&D Systems), rabbit anti-PLP (1:300; Santa Cruz Biotechnology), rabbit anti-GFAP (1:500; Dako) or rabbit anti-PDGFRα (1:300; Santa Cruz Biotechnology) diluted in 1% donkey serum/PBS, overnight at 4°C. Cells were rinsed in PBS, incubated with anti-rabbit-TRITC (tetramethylrhodamine β-isothiocyanate, 1:200; Southern Biotechnology) and anti-mouse-FITC (1:200; Sigma) for 2 h at 37°C, rinsed in PBS, incubated with DAPI (at 1:500) in PBS for 10 min, rinsed in PBS, and then mounted on coverslips.

Data analysis

Quantification of the cell numbers in Figure 2 was performed manually, and in Figures 4 and 6 performed using Zeiss Axiovision version 4.5. Comparisons between groups were made using a Student's unpaired *t* test. Comparisons of the number of stained cells in Figure 2 was done using χ^2 analysis. Comparison of clinical signs in WT (wild-type) compared with PPARδ-null mice was performed by two-way repeated measures ANOVA using data from day 25 (the start of treatment) to the end of the study (day 49). Values are means S.E.M., and for all comparisons significance was taken at P 0.05.

RESULTS

PPARδ mediates protective effects of GW0742

We have previously shown that treatment of C57BL/6 mice with the PPARδ agonist GW0742 ameliorated clinical and histological signs of EAE when administered to mice with moderate disease severity (Polak et al., 2005). To confirm that these effects were mediated via activation of PPARδ, and not by off-target actions of the agonist, we tested whether GW0742 influenced the course of disease in PPARδ-null mice in which endogenous PPARδ is inactivated in all cells and tissues by insertion of the neomycin gene into the DNA-binding domain. As previously observed, providing GW0742 to WT mice at 25 days after immunization (at which time they show moderate clinical signs) significantly reduced clinical signs beginning approx. 15 days later (Figure 1A). Immunization of PPARδ-null mice with MOG peptide resulted in a similar disease incidence and severity as WT mice, suggesting that PPARδ does not play a significant role in the early stages of EAE. However, in contrast with the WT mice, treatment with GW0742 did not effect disease progression for up to 25 days of treatment (Figure 1B). This provides strong evidence that the effects of GW0742 are mediated through this receptor and are not due to off-target actions. Since the receptor is inactivated in all cells throughout the body, these results do not allow us to conclude whether the loss of GW0742 benefit is due to lack of PPARδ from brain, or from some other tissue; however, our previous studies did not reveal any effect of GW0742 on splenic T-cells, suggesting that lack of PPARδ from brain may account for the current findings.

Effects of GW0742 on OPC maturation

We hypothesized that GW0742 could provide benefit in EAE involving effects on OL maturation or survival. To address this, we first tested whether GW0742 influenced maturation of OPCs generated from E13 mice. These preparations are grown as neurospheres, then grown on PDL-coated plates in medium containing bFGF and PDGF which leads to differentiation (Pedraza et al., 2008). After 7 days growth on PDL-coated slides, OPCs show little staining for MBP (Figure 2A)

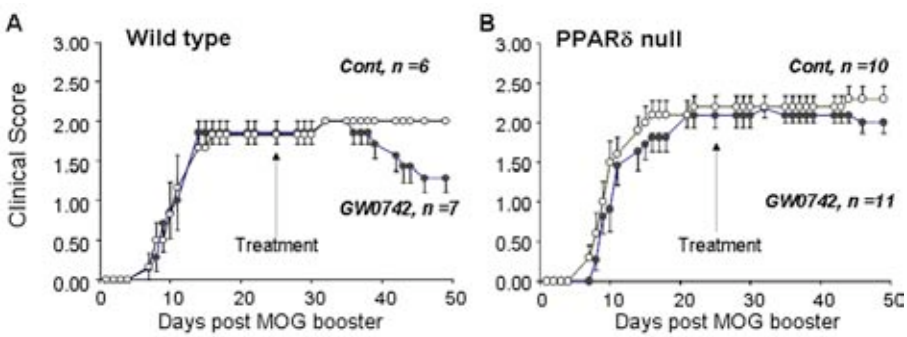

Figure 1 **GW0742 does not reduce EAE symptoms in PPARδ-null mice**
C57Bl/6 control mice (**A**) and PPARδ-null (**B**) mice were immunized with MOG_{35-55} peptide and clinical scores monitored to 50 days. On day 25 the mice were split into two subgroups having comparable disease progression (the two subgroups are indicated by the open and closed circles) and were provided free access to chow containing 0, control (○) or 100 p.p.m. GW0742 (●). Values are means S.E.M. of clinical scores. The incidence of disease reached 100% in all groups by day 14; there was no difference in the average day of onset (9.3 0.4 compared with 10.8 0.9, null compared with WT; means S.E.M.). In the WT mice, there was a statistically significant effect of GW0742 on clinical scores over time (two-way repeated measures ANOVA of day 25–49 scores, $F[14,1]$ 2.75, P 0.0012). Cont, control.

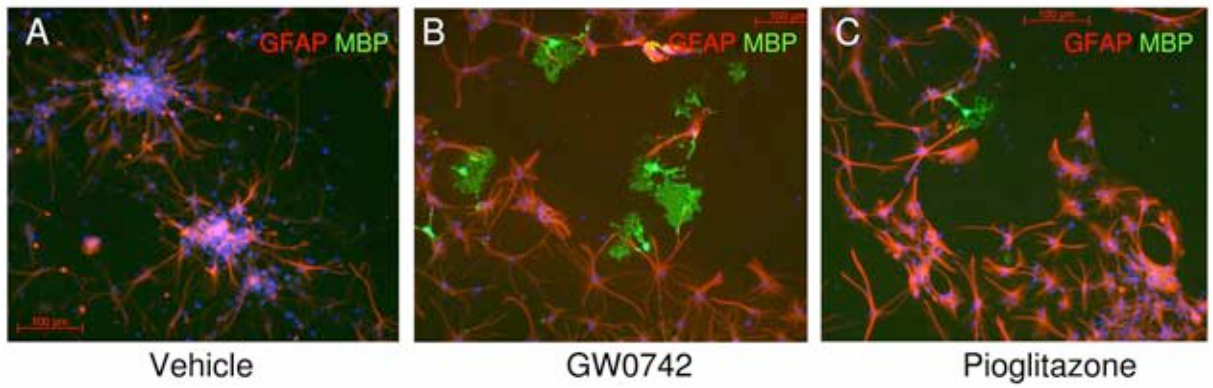

Figure 2 **Effect of GW0742 on OPC maturation**
Mouse E13 OPCs were plated on to PDL-coated coverslips, grown for 48 h in differentiation medium, medium replaced with Sato–Bottenstein medium, and cells grown for a further 5 days in the absence of growth factors and in the presence of (**A**) vehicle, (**B**) 10 μM GW0742 or (**C**) 10 μM pioglitazone. Cells were fixed and stained for GFAP (TRITC, red), MBP (FITC, green) and nuclei [DAPI (4 ,6-diamidino-2-phenylindole), blue]. Images shown are representative fields of view from three or four slides examined per condition. There was a significant increase in the number of GFAP cells from 130/993 (13.0%) in vehicle-treated, to 140/643 (25.5%) in GW0742-treated cultures (P 0.0001, as measured using a χ^2 test), but not in the pioglitazone-treated cultures (119/742; 16.1%). The number of MBP cells was significantly increased from none in the vehicle-treated to 20/643 (4.0%) in the GW0742-treated cells (P 0.0001), but not in the pioglitazone-treated cultures (4/742; 0.5%).

and the presence of numerous GFAP -stained cells. After 5 days growth in GW0742, the number of GFAP-stained cells was significantly increased from 13% (in vehicle-treated cultures) to 26% (P 0.0001, as measured using a χ^2 test); and their morphology more closely resembled that of mature astrocytes. In these cultures we also observed an increased number of cells stained for MBP, which increased from none in vehicle-treated cultures to 4% in the treated cultures (P 0.0001, as measured using a χ^2 test), as well as the appearance of myelin sheaths and longer processes (Figure 2B). Treatment with the PPARγ agonist pioglitazone did not significantly increase the number of GFAP -stained cells (16% of all cells, P 0.05 compared with vehicle), although they again showed a more mature phenotype; very few cells (0.5% of all cells, P 0.07) showed positive staining for MBP (Figure 2C).

To look at the initial events involved in the effects of GW0742 on OPC maturation, we measured mRNA levels of known markers of OPC maturation (Figure 3). After treatment of E13 OPCs with 3 μM GW0742 for 24 h, there was a significant increase in expression of *Olig1*, *CGT*, *PDGFRα* and an increase in *GalC* compared with vehicle-treated cells (Figures 3A–3D). However, at this timepoint there was no change in *PLP* mRNA levels (Figure 3E). Levels of *PPARδ* were also significantly increased by GW0742 (Figure 3F), as were levels of the well-characterized PPARδ target gene *ANGPTL-4*, although interestingly, not of a second target gene *UCP3* (Figures 3G and 3H).

Staining for the early OPC marker PDGFRα confirmed that treatment with GW0742 for 24 h increased OPC maturation as indicated by an increase in cell migration (Figure 4). GW0742 did not modify the total number of spheres present; however, the average size of the spheres was significantly reduced (diameter of 40.7 2.1 μm compared with 30.4 1.6 μm, P 0.0005) (Figures 4A and 4B). At the same time, the total number of cells that migrated out from the spheres was significantly increased by GW0742 (814 38 compared with 1135 105 cells per field; DMSO compared with GW0742, P 0.05) (Figures 4C and 4D), and those cells showed a greater number of processes (Figures 4E and 4F).

PPARδ mediates OPC maturation

To determine whether endogenous PPARδ plays a role in normal OL maturation, we prepared primary OPCs from E13 WT and PPARδ-null mice and examined process formation under full differentiation conditions (e.g. medium containing PDGFα, bFGF and B27 supplement). After 7 days, WT cells had more primary processes than did PPARδ-null cells (Figure 5A), and quantification of process number (Figure 5B) revealed that PPARδ-null cells had significantly fewer processes per cell (2.3 0.04) than WT cells (3.1 0.1). Analysis of process distribution (Figure 5C) showed a left shift in the average number of processes per cell in PPARδ-null cells, suggesting that process maturation was not completely inhibited, but either temporally delayed or limited to fewer processes in the null cells.

GW0742 increases noggin expression

In view of reports that BMPs inhibit OL maturation (Gross et al., 1996; Hardy and Friedrich, 1996; Zhu et al., 1999; Mehler et al., 2000; Mekki-Dauriac et al., 2002; Gomes et al., 2003), we hypothesized that GW0742 might act by increasing BMP antagonist expression. Immunostaining of E13 OPCs treated for 24 h with GW0742 showed staining for the BMP antagonist noggin, primarily in the non-migrating cells which remained within spheres (Figures 6A and 6B). Quantification of cell numbers revealed no significant effect of treatment on

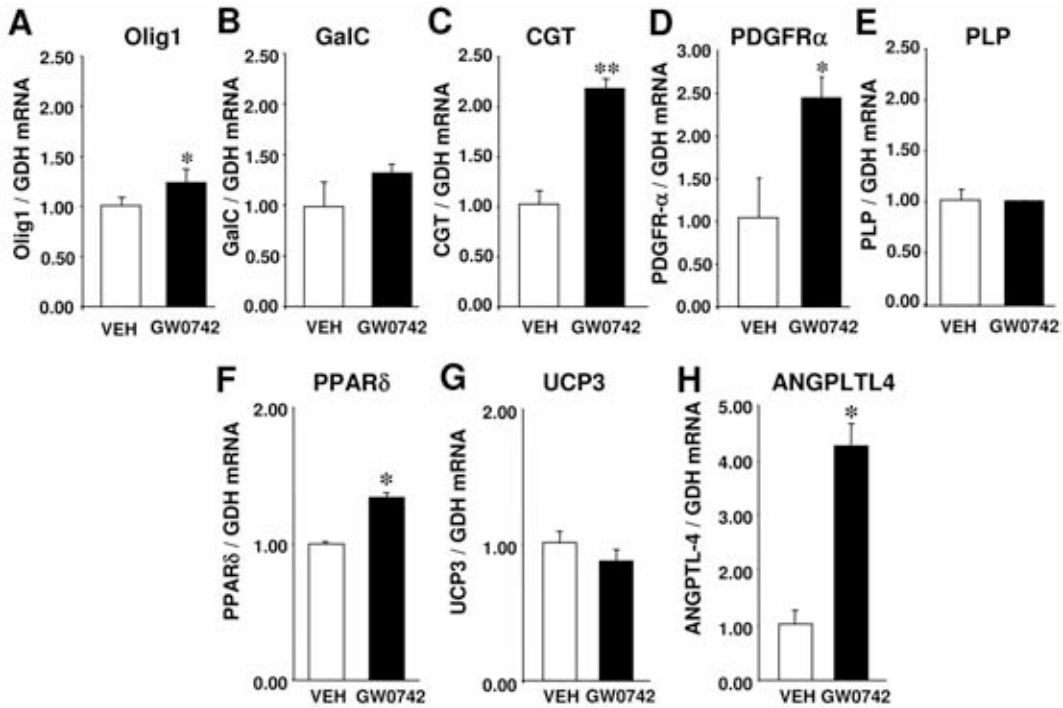

Figure 3 **Effects of GW0742 on myelin gene and PPARδ target gene expression**
Mouse E13 OPCs were plated on to PDL-coated coverslips, grown for 5 days in differentiation medium, medium replaced with Sato–Bottenstein medium, and cells grown in the absence of growth factors and in the presence of vehicle or 3 μM GW0742. After 24 h, relative levels of (**A**) *Olig1*, (**B**) *GalC*, (**C**) *CGT*, (**D**) *PDGFRα*, (**E**) *PLP*, and the PPARδ target genes (**F**) *PPARδ*, (**G**) *UCP3*; and (**H**) *ANGPTL-4* mRNAs were determined by qPCR. Values are shown as means S.E.M. of n 3 samples in each group, normalized to values measured for *GDH* in the same samples; and then normalized values in vehicle treated samples are set to 1.0; *P 0.05 and **P 0.01, compared with vehicle (VEH).

the total number of spheres (20.4 3 compared with 28.0 5, average number of spheres per field, DMSO compared with GW0742, P 0.05). However, the number of noggin-stained spheres was significantly increased (from 7.4 2 to 21.4 4 per field, P 0.01). This increased number of noggin-stained spheres reflects a significant increase from 40 10% to 79 6% (P 0.01) of all spheres. We did not observe any significant co-localization of noggin with either PDGFRα (Figures 7A and 7B), PLP (Figures 7C and 7D) or GFAP (Figures 7E and 7F), suggesting that expression was restricted to immature progenitor cells. Analysis by qPCR showed that, after 24 h of treatment, GW0742 did not increase *noggin* mRNA levels, although we did observe a significant decrease in both *BMP2* and *BMP4* mRNA levels (Figure 8A).

Since there are some astrocytes present in the OPC cultures, we tested whether GW0742 influenced BMP or BMP antagonist expression in enriched astrocyte cultures. After 24 h we observed a significant increase of *noggin* mRNA levels in astrocytes (Figure 8B); interestingly, this increase appeared to be selective for noggin since mRNA levels of other BMP antagonists (*gremlin*, *follistatin* and *bambi*) were not increased (in fact *gremlin* mRNA levels were significantly reduced). In contrast with OPCs, GW0742 did not increase *PPARδ* mRNA levels (Figure 9A). Interestingly, GW0742 caused a significant increase in the PPARδ target gene UCP3, but not in ANGPTL-4. Consistent with the increase in *noggin* mRNA, we observed a large increase in noggin staining, present in vesicular structures around the nucleus of primary astrocytes treated with 3 μM GW0742 for 24 h (Figure 10).

DISCUSSION

In the present study we show that PPARδ is involved in the regulation of OPC maturation and is associated with changes in the expression of BMPs and BMP antagonists. We previously have shown that treatment of EAE-immunized mice with GW0742 did not offer significant protection when administered early during disease evolution, but instead reduced clinical signs when given to mice showing moderate clinical signs (Polak et al., 2005). In those studies, GW0742 did not suppress the ability of T-cells to produce IFNγ (interferon γ), providing a possible explanation for its inability to reduce early-stage EAE. In contrast, GW0742 significantly increased *PLP* and *MBP* mRNA levels in EAE mice, suggesting possible effects on OL maturation, survival or proliferation. The findings of the present study support this possibility since, in E13 OPCs, GW0742 induced maturation as determined by

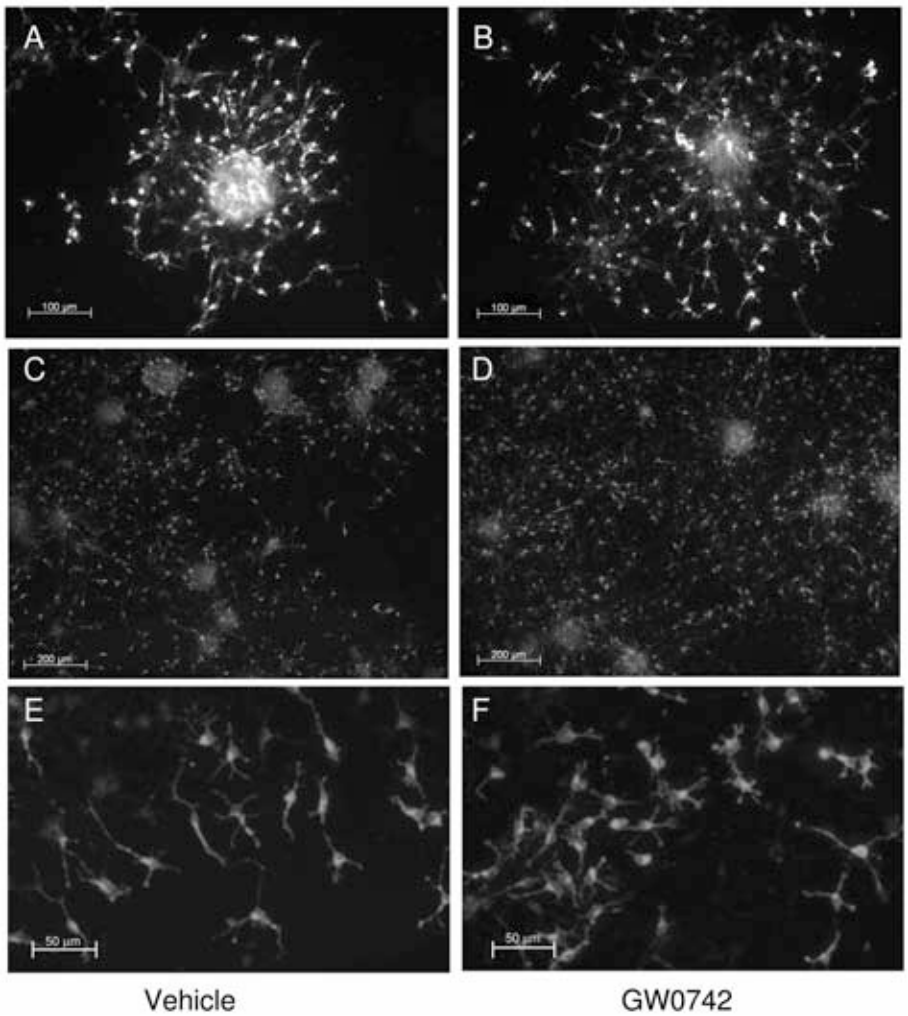

Figure 4 **Effect of GW0742 on PDGFRα expression**

Mouse E13 OPCs were plated on to PDL-coated coverslips, grown for 5 days in differentiation medium, the medium replaced with Sato–Bottenstein medium, and the cells grown for 24 h in the absence of growth factors and in the presence of vehicle (**A**, **C** and **E**) or 3 μM GW0742 (**B**, **D** and **F**). Cells were fixed, stained for PDGFRα, and cell numbers and size quantified in six fields of view taken at 20 × magnification. The total number of spheres (16.8 2.5 compared with 15 1.3 spheres per field, DMSO compared with GW0742, *P* 0.05) was not modified by treatment. (**A** and **B**) are representative images showing the smaller average size of spheres in GW0742-treated cultures (40.7 2.1 compared with 30.4 1.6 m diameter, *P* 0.0005). (**C** and **D**) are representative images showing increased numbers of PDGFRα-stained migrating cells (814 38 compared with 1135 105 cells per field, DMSO compared with GW0742, *P* 0.05). (**E** and **F**) are representative images showing increased number of processes on the migrating cells in the GW0742-treated cultures.

increased myelin gene expression, increased myelin sheets and increased numbers of pre-myelinating OPCs. The finding that mRNA levels for PLP were not increased suggests that GW0742 affects earlier stages of OPC maturation, although whether PLP is increased at later times is not yet known. Overall, these findings are in agreement with earlier results showing that the weaker PPARδ agonist bromopalmitate (Granneman et al., 1998) and the more selective agonist L-796449 (Saluja et al., 2001) induced myelin expression in post-natal mouse OPC cultures. Furthermore, the results that GW0742 was ineffective at reducing clinical signs in PPARδ-null mice confirms that the actions of this drug depend upon PPARδ and are not due to off-target effects as has been reported for other PPAR agonists (Dello Russo et al., 2003).

We examined the specificity of PPAR agonist effects by comparing the actions of GW0742 with those of the selective PPARγ agonist pioglitazone on OPC maturation (Figure 2). Increased MBP staining was seen primarily in the GW0742-treated OPCs, suggesting a more important role for PPARδ as compared with PPARγ in the OPC maturation process. Interestingly, treatment with either GW0742 or pioglitazone led to more mature astrocyte morphology in the GFAP-positive-stained cells, suggesting that both PPARs may be

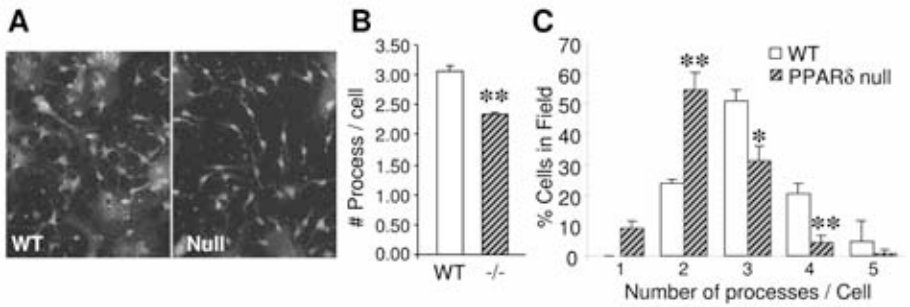

Figure 5 **PPARδ deficiency reduces OPC maturation**
(**A**) OPCs from WT and PPARδ-null mice were plated on to PDL coverslides and kept in full differentiation medium for 7 days, after which the number of primary processes per cell were counted in three different slides of each, with approx. 100 cells counted per slide. (**B**) Average number of processes per cell (a total of 300–325 cells were counted in each group). (**C**) The percentage of cells having the indicated number of processes. Values are means S.D.; **P* 0.05 and ***P* 0.005, compared with WT.

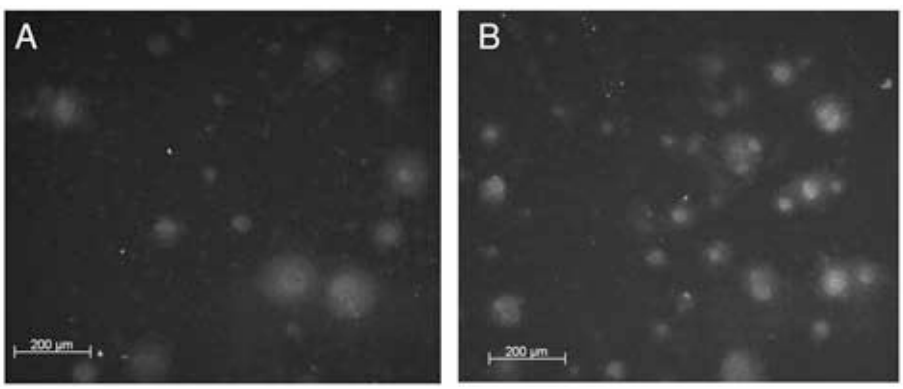

Figure 6 **GW0742 increases noggin expression in OPC cultures**
Mouse E13 OPCs were grown for 5 days in differentiation medium, then treated with (**A**) vehicle or (**B**) 3 μM GW0742 for 24 h. Cells were then fixed and immunostained for noggin. Cell counting was carried out to quantify the total number of spheres and total number of spheres stained for noggin in seven fields of view for each condition taken at 20 × magnification. There was a similar number of spheres in the two groups (20.4 3 compared with 28.0 5, average number of spheres per field, DMSO compared with GW0742, *P* 0.05). GW0742 significantly increased the number of noggin-stained spheres (from 7.4 2 to 21.4 4 per field, *P* 0.01) and the percentage of noggin-stained spheres increased from 40 10% to 79 6% (*P* 0.01). Representative images are shown for each condition.

involved in astrocyte maturation. Our findings also show that endogenous PPARδ plays a role in OPC maturation, since in PPARδ-deficient OPCs the distribution and average number of processes was significantly reduced compared with WT OPCs. It should be noted that, despite the absence of PPARδ, these OPCs still developed processes and sheaths, but OPC maturation was reduced, pointing to PPARδ as a modulator of the maturation process. PPARδ may be considered as a potential feed-forward factor along OPC maturation, even though it is not yet clear at which step through the maturation PPARδ exerts its role.

We focused attention on the class of BMPs and BMP antagonists for several reasons. Numerous studies have shown a role for BMPs in regulating neural stem cell commitment (Kondo and Raff, 2004; Gaughwin et al., 2006; Hampton et al., 2007a; Talbott et al., 2006; Cheng et al., 2007); and in restricting OPC maturation during normal development (Gross et al., 1996; Hardy and Friedrich, 1996; Zhu et al., 1999; Mehler et al., 2000; Mekki-Dauriac et al., 2002; Gomes et al., 2003). This has been thought to be a BMP-dependent induction of Id proteins that can bind to and inactivate the bHLH proteins Olig1 and Olig2 (Samanta and Kessler, 2004) which promote OPC maturation.

Recently, it was shown that in EAE, *BMP4*, *-6* and *-7* are up-regulated in lumbar spinal cord; with *BMP4* being the most abundant mRNA detected, and being detected in astrocytes as well as in oligodendrocytes and macrophages (Ara et al., 2008). In a second related study, it was found that *BMP4* and *BMP7* were increased following lysolecithin-induced demyelination, and interestingly that phosphorylated Smad 1/5/8 was detected in astrocytes (Fuller et al., 2007), suggesting that BMPs can induce astrogliosis and inhibit remyelination. Taken together, these findings raised the possibility that treatments or interventions that increase BMP

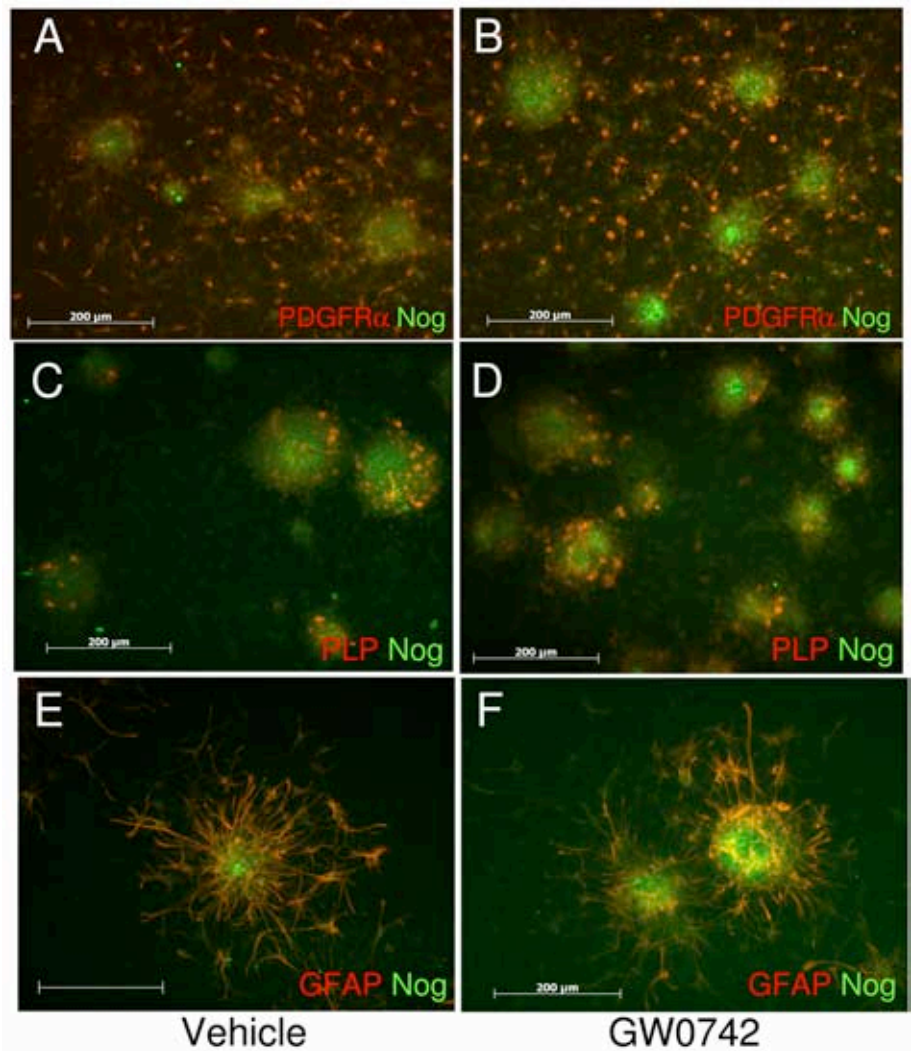

Figure 7 **Noggin does not co-localize with OPC markers**
Mouse E13 OPCs were grown for 5 days in differentiation medium, then treated for 24 h with 3 μM GW0742 or vehicle. Cells were then fixed and immunostained for noggin (FITC, green) together with PDGFRα (**A** and **B**), PLP (**C** and **D**) or GFAP (**E** and **F**). Representative images are shown for each condition. Nog, noggin.

antagonist expression, or reduce BMP expression, would facilitate OPC maturation. Several previous studies have shown that PPAR agonists can influence BMP signalling. In human umbilical vein endothelial cells, pioglitazone suppressed *BMP2* expression (Zhang et al., 2008), several PPARγ agonists decreased *BMP2* expression in human osteoblasts (Lin et al., 2007), and in mouse gonadotopinoma cells, pioglitazone reduced BMP signalling including activation of Id1 expression and DNA synthesis (Takeda et al., 2007). In the present study we extend this list by demonstrating that a PPARδ agonist can decrease BMP2 and BMP4 expression in OPCs, and to our knowledge this is the first demonstration of any PPAR agonist increasing a BMP antagonist.

Our results point to distinct effects of GW0742 on BMPs and their antagonists' in astrocytes and OPCs. In OPCs GW0742 primarily affected expression of *BMP2* and *BMP4* mRNAs with lesser effects on the BMP antagonists; in astrocytes the PPARδ agonist had greater effects on expression of the BMP antagonists noggin and gremlin, with smaller effects on BMPs. The lack of significant changes in *BMP2* and *BMP4* expression in GW0742-treated astrocytes, together with down-regulation of the same BMPs in GW0742-treated OPCs, suggests the possibility of an autocrine short-range effect of BMPs in astrocytes and OPCs. This may be similar to the balance of interactions between BMPs and their antagonists that occur in the optic nerve which result in enhanced OPC maturation (Kondo and Raff, 2004). This may guarantee cellular identity for astrocytes and precursor cell identity for OPCs. On the other hand, increase in noggin expression in astrocytes following GW0742

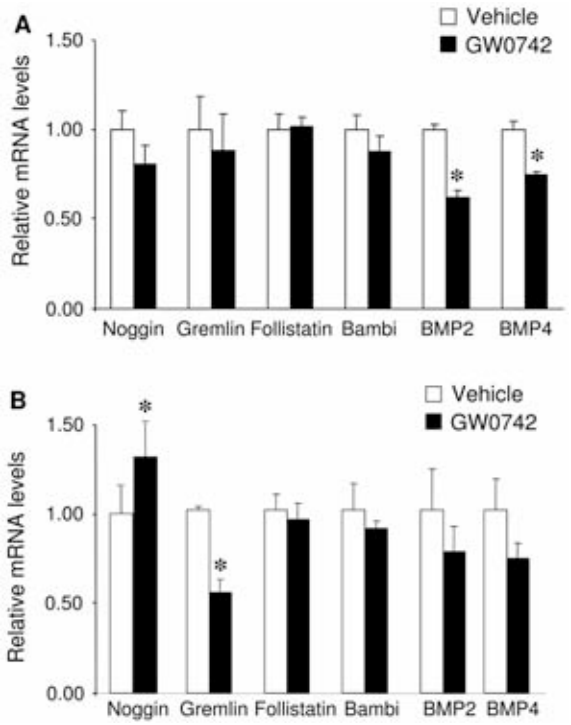

Figure 8 Regulation of BMP and BMP antagonist expression by GW0742
Mouse E13 OPCs (**A**) grown for 5 days in full medium and primary rat astrocytes (**B**) were treated with 3 μM GW0742 or vehicle for 24 h, and then RNA was isolated and qPCR used to measure relative mRNA levels of indicated genes. Values are means S.E.M. of the relative mRNA level normalized to values for *GDH* measured in the same samples, *n* 3. The mRNA values for the vehicle groups were set to 1.0; **P* 0.05, compared with the corresponding vehicle treated sample (as measured using an unpaired Student's *t* test).

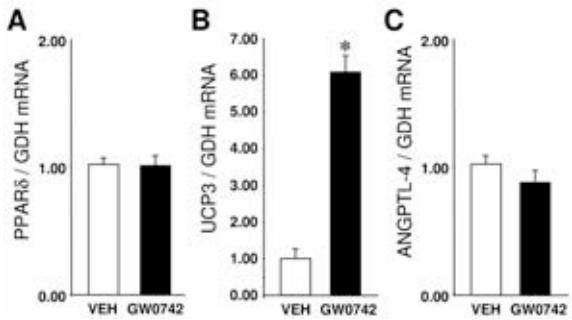

Figure 9 Regulation of PPARδ target gene expression in astrocytes
Primary rat astrocytes were treated with 3 μM GW0742 or vehicle (VEH) for 24 h then RNA isolated and qPCR used to measure relative mRNA levels of (**A**) PPARδ and its target genes, (**B**) *UCP3* and (**C**) *ANGPTL-4*. Values are means S.E.M. of the relative mRNA level normalized to values for *GDH* measured in the same samples, *n* 3. The mRNA values for the vehicle groups were set to 1.0; **P* 0.05, compared with the corresponding vehicle-treated sample (as measured using an unpaired Student's *t* test).

treatment could represent a modulatory mechanism of the intercellular communication between astrocyte and OPCs, and may suggest a PPARδ-mediated role of astrocytes in OPC maturation.

At the protein level we observed in both OPCs and astrocytes that GW0742 treatment increased staining for noggin protein, although the increase was more robust in the primary astrocyte cultures. Whereas increased staining could be due to an overall increase in noggin expression, the fact that noggin is normally released raises the possibility that GW0742 reduced release from astrocytes. If so, the absence of strong intracellular noggin staining in the OPCs could be due to increased release owing to GW0742, suggesting cell-specific means of regulating release. The greater increase in primary astrocyte cultures compared with the astrocytes present in the OPC cultures could also be due to differences in the maturation state of astrocytes, suggesting that only more mature cells can highly express noggin.

Previous characterization of BMPs and BMP antagonists in astrocytes is limited. In optic nerve astrocytes, the mRNAs for *gremlin*, *follistatin*, *chordin* and *bambi*, but not *noggin*, were detected (Wordinger et al., 2002); and *noggin* mRNA was expressed in type 1 astrocytes in P6 rat optic nerve (Kondo and Raff, 2004). Immunohistochemical staining demonstrated that noggin was primarily expressed in astrocytes in the dorsal spinal cord following rhizotomy, but was absent from non-injured spinal cords (Hampton et al., 2007b).

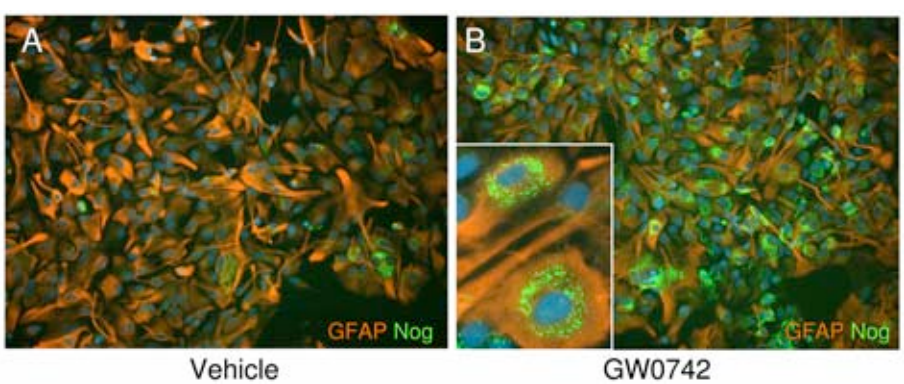

Figure 10 **GW0742 increases noggin expression in astrocytes**
Primary rat astrocytes were treated for 24 h with 3 μM GW0742 or vehicle, after which cells were fixed and immunostained for noggin (Nog) and GFAP.

Astrocytes have been shown to express *BMP4* in the adult rat CNS (Mikawa et al., 2006), *BMP4* and *7* primarily in olfactory bulb astrocytes throughout development (Peretto et al., 2002), and the mRNAs for *BMP2, 4, 5* and *7* in cultured adult optic head astrocytes (Wordinger et al., 2002). It has also been shown that BMP2/4 is increased in ischaemic astrocytes (Xin et al., 2006), and that the mRNA levels of *BMP4*, but not *BMP2*, are increased in astrocytes after spinal cord injury (Chen et al., 2005), suggesting regulation following injury. These indications that BMP levels are increased under pathological conditions are consistent with our findings that a treatment to reduce pathology leads to a reduction in astroglial BMP levels.

The findings in the present study that agonists of PPARδ can down-regulate the BMP signalling system may be of particular relevance during diseases such as EAE since inflammatory conditions have been shown to increase BMP signalling. For example, in prostate cancer cells, NF-κB (nuclear factor κB) binds to the *BMP2* promoter and induces *BMP2* expression, and in chondrocytes, TNFα (tumour necrosis factor α) induces *BMP2* (Fukui et al., 2006), most probably by also binding to the *BMP2* promoter (Feng et al., 2003). It is known that certain cytokines including TNFα and IFNγ, which are present in the EAE brain, cause reversible inhibition of OPC proliferation and maturation (Agresti et al., 1996), and that neurogenesis is sensitive to the inflammatory milieu and that chronic inflammation can reduce neurogenesis (Monje et al., 2003; Pluchino et al., 2008; Ekdahl et al., 2009; Whitney et al., 2009). Therefore, in addition to direct effects on BMPs and BMP antagonists, it is likely that PPARδ down-regulates BMP signalling by attenuating inflammatory activation, as it has been shown to do in different cells and tissues (Delerive et al., 1999; Planavila et al., 2005; Kilgore and Billin, 2008; Smeets et al., 2008).

In summary, the results of the present study confirm that PPARδ mediates the effects of the synthetic agonist GW0742 in EAE and also plays a role in normal OPC maturation. Treatment with GW0742 regulates both BMP, as well as BMP antagonist, expression in astrocytes and OPCs, although with distinct effects. The molecular mechanisms underlying the ability of PPARδ agonists to modulate BMP and BMP antagonist regulation could involve both direct effects on transcription via binding to PPAR-responsive elements, and indirect effects due to anti-inflammatory actions which could reduce inflammatory up-regulation of BMPs. The recent demonstration that PPARγ agonists can be protective in relapsing/remitting MS patients (Kaiser et al., 2009), together with the observations that PPARδ agonists promote OPC maturation, suggests that clinical trials of pure or mixed PPAR agonists may be of therapeutic value in the treatment of MS.

ACKNOWLEDGEMENTS

We thank Dr Tim Willson for providing GW0742, and Anthony Sharp and Shao Xia-Lin for assistance with animal care and preparation of primary cell cultures.

FUNDING

This work was supported, in part, by the National Multiple Sclerosis Society [grant number PP1460].

REFERENCES

Agresti C, D'Urso D, Levi G (1996) Reversible inhibitory effects of interferon-γ and tumour necrosis factor-α on oligodendroglial lineage cell proliferation and differentiation in vitro. Eur J Neurosci 8:1106–1116.

Ara J, See J, Mamontov P, Hahn A, Bannerman P, Pleasure D, Grinspan JB (2008) Bone morphogenetic proteins 4, 6, and 7 are up-regulated in mouse spinal cord during experimental autoimmune encephalomyelitis. J Neurosci Res 86:125–135.

Basu-Modak S, Braissant O, Escher P, Desvergne B, Honegger P, Wahli W (1999) Peroxisome proliferator-activated receptor β regulates acyl-CoA synthetase 2 in reaggregated rat brain cell cultures. J Biol Chem 274:35881–35888.

Bilican B, Fiore-Heriche C, Compston A, Allen ND, Chandran S (2008) Induction of Olig2 precursors by FGF involves BMP signalling blockade at the Smad level. PLoS ONE 3:e2863.

Chen J, Leong SY, Schachner M (2005) Differential expression of cell fate determinants in neurons and glial cells of adult mouse spinal cord after compression injury. Eur J Neurosci 22:1895–1906.

Cheng X, Wang Y, He Q, Qiu M, Whittemore SR, Cao Q (2007) Bone morphogenetic protein signaling and olig1/2 interact to regulate the differentiation and maturation of adult oligodendrocyte precursor cells. Stem Cells 25:3204–3214.

Dasgupta S, Roy A, Jana M, Hartley DM, Pahan K (2007) Gemfibrozil ameliorates relapsing-remitting experimental autoimmune encephalomyelitis independent of peroxisome proliferator-activated receptor-α. Mol Pharmacol 72:934–946.

Delerive P, De BK, Besnard S, Vanden BW, Peters JM, Gonzalez FJ, Fruchart JC, Tedgui A, Haegeman G, Staels B (1999) Peroxisome proliferator-activated receptor α negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1. J Biol Chem 274:32048–32054.

Dello Russo C, Gavrilyuk V, Weinberg G, Almeida A, Bolanos JP, Palmer J, Pelligrino D, Galea E, Feinstein DL (2003) Peroxisome proliferator-activated receptor γ thiazolidinedione agonists increase glucose metabolism in astrocytes. J Biol Chem 278:5828–5836.

Deplanque D, Gele P, Petrault O, Six I, Furman C, Bouly M, Nion S, Dupuis B, Leys D, Fruchart JC, Cecchelli R, Staels B, Duriez P, Bordet R (2003) Peroxisome proliferator-activated receptor-α activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment. J Neurosci 23:6264–6271.

Diab A, Deng C, Smith JD, Hussain RZ, Phanavanh B, Lovett-Racke AE, Drew PD, Racke MK (2002) Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-Δ(12,14)-prostaglandin J(2) ameliorates experimental autoimmune encephalomyelitis. J Immunol 168:2508–2515.

Drew PD, Xu J, Storer PD, Chavis JA, Racke MK (2006) Peroxisome proliferator-activated receptor agonist regulation of glial activation: relevance to CNS inflammatory disorders. Neurochem Int 49:183–189.

Ekdahl CT, Kokaia Z, Lindvall O (2009) Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158:1021–1029.

Feinstein DL, Galea E, Gavrilyuk V, Brosnan CF, Whitacre CC, Dumitrescu-Ozimek L, Landreth GE, Pershadsingh HA, Weinberg G, Heneka MT (2002) Peroxisome proliferator-activated receptor-γ agonists prevent experimental autoimmune encephalomyelitis. Ann Neurol 51:694–702.

Feinstein DL (2003) Therapeutic potential of peroxisome proliferator-activated receptor agonists for neurological disease. Diabetes Technol Ther 5:67–73.

Feng JQ, Xing L, Zhang JH, Zhao M, Horn D, Chan J, Boyce BF, Harris SE, Mundy GR, Chen D (2003) NF-κB specifically activates BMP-2 gene expression in growth plate chondrocytes *in vivo* and in a chondrocyte cell line *in vitro*. J Biol Chem 278:29130–29135.

Fukui N, Ikeda Y, Ohnuki T, Hikita A, Tanaka S, Yamane S, Suzuki R, Sandell LJ, Ochi T (2006) Pro-inflammatory cytokine tumor necrosis factor-α induces bone morphogenetic protein-2 in chondrocytes via mRNA stabilization and transcriptional up-regulation. J Biol Chem 281:27229–27241.

Fuller ML, DeChant AK, Rothstein B, Caprariello A, Wang R, Hall AK, Miller RH (2007) Bone morphogenetic proteins promote gliosis in demyelinating spinal cord lesions. Ann Neurol 62:288–300.

Gaughwin PM, Caldwell MA, Anderson JM, Schwiening CJ, Fawcett JW, Compston DA, Chandran S (2006) Astrocytes promote neurogenesis from oligodendrocyte precursor cells. Eur J Neurosci 23:945–956.

Gomes WA, Mehler MF, Kessler JA (2003) Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. Dev Biol 255:164–177.

Goumans MJ, Mummery C (2000) Functional analysis of the TGFβ receptor/Smad pathway through gene ablation in mice. Int J Dev Biol 44:253–265.

Granneman J, Skoff R, Yang X (1998) Member of the peroxisome proliferator-activated receptor family of transcription factors is differentially expressed by oligodendrocytes. J Neurosci Res 51:563–573.

Gross RE, Mehler MF, Mabie PC, Zang Z, Santschi L, Kessler JA (1996) Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron 17:595–606.

Hampton DW, Asher RA, Kondo T, Steeves JD, Ramer MS, Fawcett JW (2007a) A potential role for bone morphogenetic protein signalling in glial cell fate determination following adult central nervous system injury in vivo. Eur J Neurosci 26:3024–3035.

Hampton DW, Steeves JD, Fawcett JW, Ramer MS (2007b) Spinally upregulated noggin suppresses axonal and dendritic plasticity following dorsal rhizotomy. Exp Neurol 204:366–379.

Hardy RJ, Friedrich Jr, VL (1996) Oligodendrocyte progenitors are generated throughout the embryonic mouse brain, but differentiate in restricted foci. Development 122:2059–2069.

Izrael M, Zhang P, Kaufman R, Shinder V, Ella R, Amit M, Itskovitz-Eldor J, Chebath J, Revel M (2007) Human oligodendrocytes derived from embryonic stem cells: effect of noggin on phenotypic differentiation *in vitro* and on myelination *in vivo*. Mol Cell Neurosci 34:310–323.

Kaiser CC, Shukla DK, Stebbins GT, Skias DD, Jeffery DR, Stefoski D, Katsamakis G, Feinstein DL (2009) A pilot test of pioglitazone as an add-on in patients with relapsing remitting multiple sclerosis. J Neuroimmunol 211:124–130.

Kielian T, Drew PD (2003) Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation. J Neurosci Res 71:315–325.

Kilgore KS, Billin AN (2008) PPARβ/δ ligands as modulators of the inflammatory response. Curr Opin Investig Drugs 9:463–469.

Kliewer SA, Xu HE, Lambert MH, Willson TM (2001) Peroxisome proliferator-activated receptors: from genes to physiology. Recent Prog Horm Res 56:239–263.

Klotz L, Diehl L, Dani I, Neumann H, von Oppen N, Dolf A, Endl E, Klockgether T, Engelhardt B, Knolle P (2007) Brain endothelial PPARγ controls inflammation-induced CD4 T cell adhesion and transmigration *in vitro*. J Neuroimmunol 190:34–43.

Kondo T, Raff MC (2004) A role for Noggin in the development of oligodendrocyte precursor cells. Dev Biol 267:242–251.

Lin TH, Yang RS, Tang CH, Lin CP, Fu WM (2007) PPARγ inhibits osteogenesis via the down-regulation of the expression of COX-2 and iNOS in rats. Bone 41:562–574.

Lovett-Racke AE, Hussain RZ, Northrop S, Choy J, Rocchini A, Matthes L, Chavis JA, Diab A, Drew PD, Racke MK (2004) Peroxisome proliferator-activated receptor α agonists as therapy for autoimmune disease. J Immunol 172:5790–5798.

Mabie PC, Mehler MF, Kessler JA (1999) Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. J Neurosci 19:7077–7088.

Mehler MF, Mabie PC, Zhang D, Kessler JA (1997) Bone morphogenetic proteins in the nervous system. Trends Neurosci 20:309–317.

Mehler MF, Mabie PC, Zhu G, Gokhan S, Kessler JA (2000) Developmental changes in progenitor cell responsiveness to bone morphogenetic proteins differentially modulate progressive CNS lineage fate. Dev Neurosci 22:74–85.

Mekki-Dauriac S, Agius E, Kan P, Cochard P (2002) Bone morphogenetic proteins negatively control oligodendrocyte precursor specification in the chick spinal cord. Development 129:5117–5130.

Michalik L, Desvergne B, Wahli W (2003) Peroxisome proliferator-activated receptors β/δ: emerging roles for a previously neglected third family member. Curr Opin Lipidol 14:129–135.

Mikawa S, Wang C, Sato K (2006) Bone morphogenetic protein-4 expression in the adult rat brain. J Comp Neurol 499:613–625.

Miller DH, MacManus DH, Miszkiel KA, Giovannoni G, Young C (2005) An exploratory study of rosiglitazone maleate in relapsing-remitting multiple sclerosis. Multiple Sclerosis 11:S164.

Miyazono K, Miyazawa K (2002) Id: a target of BMP signaling. Sci STKE 2002:E40.

Monje ML, Toda H, Palmer TD (2003) Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760–1765.

Natarajan C, Bright JJ (2002) Peroxisome proliferator-activated receptor-γ agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation. Genes Immun 3:59–70.

Pedraza CE, Monk R, Lei J, Hao Q, Macklin WB (2008) Production, characterization, and efficient transfection of highly pure oligodendrocyte precursor cultures from mouse embryonic neural progenitors. Glia 56:1339–1352.

Peretto P, Cummings D, Modena C, Behrens M, Venkatraman G, Fasolo A, Margolis FL (2002) BMP mRNA and protein expression in the developing mouse olfactory system. J Comp Neurol 451:267–278.

Peters JM, Lee SS, Li W, Ward JM, Gavrilova O, Everett C, Reitman ML, Hudson LD, Gonzalez FJ (2000) Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor $\beta(\delta)$. Mol Cell Biol 20:5119–5128.

Planavila A, Rodriguez-Calvo R, Jove M, Michalik L, Wahli W, Laguna JC, Vazquez-Carrera M (2005) Peroxisome proliferator-activated receptor β/δ activation inhibits hypertrophy in neonatal rat cardiomyocytes. Cardiovasc Res 65:832–841.

Pluchino S, Muzio L, Imitola J, Deleidi M, faro-Cervello C, Salani G, Porcheri C, Brambilla E, Cavasinni F, Bergamaschi A, Garcia-Verdugo JM, Comi G, Khoury SJ, Martino G (2008) Persistent inflammation alters the function of the endogenous brain stem cell compartment. Brain 131:2564–2578.

Polak PE, Kalinin S, Dello RC, Gavrilyuk V, Sharp A, Peters JM, Richardson J, Willson TM, Weinberg G, Feinstein DL (2005) Protective effects of a peroxisome proliferator-activated receptor-β/δ agonist in experimental autoimmune encephalomyelitis. J Neuroimmunol 168:65–75.

Rosenberger TA, Hovda JT, Peters JM (2002) Targeted disruption of peroxisomal proliferator-activated receptor β (δ) results in distinct gender differences in mouse brain phospholipid and esterified FA levels. Lipids 37:495–500.

Saluja I, Granneman JG, Skoff RP (2001) PPARδ agonists stimulate oligodendrocyte differentiation in tissue culture. Glia 33:191–204.

Samanta J, Kessler JA (2004) Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. Development 131:4131–4142.

See JM, Grinspan JB (2009) Sending mixed signals: bone morphogenetic protein in myelination and demyelination J. Neuropathol Exp Neurol 68:595–604.

Smeets PJ, Teunissen BE, Planavila A, de Vogel-van den Bosch H, Willemsen PH, van der Vusse GJ, van Bilsen M (2008) Inflammatory pathways are activated during cardiomyocyte hypertrophy and attenuated by peroxisome proliferator-activated receptors PPARα and PPARδ. J Biol Chem 283:29109–29118.

Sznaidman ML, Haffner CD, Maloney PR, Fivush A, Chao E, Goreham D, Sierra ML, LeGrumelec C, Xu HE, Montana VG, Lambert MH, Willson TM, Oliver WR, Sternbach DD (2003) Novel selective small molecule agonists for peroxisome proliferator-activated receptor δ (PPARδ)-synthesis and biological activity. Bioorg Med Chem Lett 13:1517–1521.

Takeda M, Otsuka F, Otani H, Inagaki K, Miyoshi T, Suzuki J, Mimura Y, Ogura T, Makino H (2007) Effects of peroxisome proliferator-activated receptor activation on gonadotropin transcription and cell mitosis induced by bone morphogenetic proteins in mouse gonadotrope LβT2 cells. J Endocrinol 194:87–99.

Talbott JF, Cao Q, Enzmann GU, Benton RL, Achim V, Cheng XX, Mills MD, Rao MS, Whittemore SR (2006) Schwann cell-like differentiation by adult oligodendrocyte precursor cells following engraftment into the demyelinated spinal cord is BMP-dependent. Glia 54:147–159.

ten Dijke P, Korchynskyi O, Valdimarsdottir G, Goumans MJ (2003) Controlling cell fate by bone morphogenetic protein receptors. Mol Cell Endocrinol 211:105–113.

Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC (2009) Inflammation mediates varying effects in neurogenesis: Relevance to the pathogenesis of brain injury and neurodegenerative disorders. J Neurochem 108:1343–1359.

Wordinger RJ, Agarwal R, Talati M, Fuller J, Lambert W, Clark AF (2002) Expression of bone morphogenetic proteins (BMP), BMP receptors, and BMP associated proteins in human trabecular meshwork and optic nerve head cells and tissues. Mol Vis 8:241–250.

Xin H, Li Y, Chen X, Chopp M (2006) Bone marrow stromal cells induce BMP2/4 production in oxygen-glucose-deprived astrocytes, which promotes an astrocytic phenotype in adult subventricular progenitor cells. J Neurosci Res 83:1485–1493.

Xu J, Storer PD, Chavis JA, Racke MK, Drew PD (2005) Agonists for the peroxisome proliferator-activated receptor-α and the retinoid X receptor inhibit inflammatory responses of microglia. J Neurosci Res 81:403–411.

Xu J, Chavis JA, Racke MK, Drew PD (2006) Peroxisome proliferator-activated receptor-α and retinoid X receptor agonists inhibit inflammatory responses of astrocytes. J Neuroimmunol 176:95–105.

Xu J, Racke MK, Drew PD (2007) Peroxisome proliferator-activated receptor-α agonist fenofibrate regulates IL-12 family cytokine expression in the CNS: relevance to multiple sclerosis. J Neurochem 103:1801–1810.

Yanagita M (2005) BMP antagonists: their roles in development and involvement in pathophysiology. Cytokine Growth Factor Rev 16:309–317.

Zhang M, Zhou SH, Zhao S, Li XP, Liu LP, Shen XQ (2008) Pioglitazone can downregulate bone morphogenetic protein-2 expression induced by high glucose in human umbilical vein endothelial cells. Pharmacology 81:312–316.

Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH (1999) A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. Nature 400:687–693.

Received 19 June 2009/7 December 2009; accepted 9 December 2009

Published as Immediate Publication 9 December 2009, doi 10.1042/AN20090033