Published in final edited form as:

Am J Physiol Gastrointest Liver Physiol. 2007 February ; 292(2): G657–G666.

Expression of Peroxisome Proliferator-Activated Receptor- γ in Macrophage Suppresses Experimentally-Induced Colitis

Yatrik M Shah¹, Keiichirou Morimura¹, and Frank J Gonzalez^{1,2}

¹Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Abstract

Objectives—Peroxisome proliferator-activated receptor γ (PPAR γ) has been shown to be a protective transcription factor in mouse models of inflammatory bowel disease (IBD). PPAR γ is expressed in several different cell types, and mice with a targeted disruption of the PPAR γ gene in intestinal epithelial cells demonstrated increased susceptibility to dextran sodium sulfate (DSS)-induced IBD. However, the highly selective PPAR γ ligand rosiglitazone decreased the severity of DSS-induced colitis and suppressed cytokine production in both PPAR γ intestinal specific null mice and wild-type littermates. Therefore the role of PPAR γ in different tissues and their contribution to the pathogenesis of IBD still remain unclear.

Methods—Mice with a targeted disruption of PPAR γ in macrophages (PPAR $\gamma^{\Delta M\phi}$) and wild-type littermates (PPAR $\gamma^{F/F}$) were administered 2.5% DSS in drinking water to induce IBD. Typical clinical symptoms were evaluated on a daily basis and proinflammatory cytokine analysis was performed.

Results—PPAR $\gamma^{\Delta M\phi}$ mice displayed an increased susceptibility to DSS-induced colitis in comparison to wild-type littermates, as defined by body weight loss, diarrhea, rectal bleeding score, colon length and histology. Interleukin (IL)-1 β , CCR2, MCP-1 and iNOS mRNA levels in colons of PPAR $\gamma^{\Delta M\phi}$ mice treated with DSS were higher than in similarly treated PPAR $\gamma^{F/F}$ mice.

Conclusions—The present study has identified a novel protective role for macrophage PPAR γ in the DSS-induced IBD model. The data suggest that PPAR γ regulates recruitment of macrophages to inflammatory foci in the colon.

Keywords

CC chemokine receptor 2; Peroxisome Proliferator-Activated Receptor; Macrophages; Colitis

Introduction

Peroxisome proliferator-activated receptor γ (PPAR γ) is a member of the nuclear receptor superfamily of transcription factors. Following activation of PPAR γ by specific ligands, the receptor binds to its obligate heterodimer partner retinoid X receptor (RXR). Ligand binding induces a conformational change resulting in the release of corepressors, binding of a distinct set of nuclear coactivators, and activation of gene transcription [47]. In addition to endogenous fatty acids and their derivatives [26,27], PPAR γ binds to certain class of insulin-sensitizing agents known as the thiazolidinediones (TZDs) [30]. Two commonly used TZDs, rosiglitazone and pioglitazone bind with high affinity to PPAR γ and are in widespread clinical use for anti-type 2 diabetes therapy. [34] PPAR γ is highly expressed in brown and white adipose tissue,

²Correspondence to: Frank J. Gonzalez, Building 37, Room 3106, National Cancer Institute, Bethesda, Maryland 20892, USA. Phone: (301) 496-9067; Fax: (301) 496-8419; E-Mail: fgonz@helix.nih.gov.

and shown to be a key transcription factor in adipocyte differentiation [37,40]. Transgenic mice containing a conditional disruption of PPAR γ in adipocytes displayed a severe reduction in brown and white adipocyte cell mass [21,23,24].

More recently, PPAR γ was shown to be critical in inflammatory bowel disease (IBD) [1,5,17,25,31,38,42,44]. IBD, which manifests as either ulcerative colitis (UC) or Crohn's disease (CD), is associated with chronic inflammation of the intestinal tract. PPAR γ ligands can attenuate the severity of mouse models of IBD induced by dextran sodium sulfate (DSS) or 2,4,6-trinitrobenzenesulphonic acid (TNBS) [5,17,25,31,38,42,44]. Furthermore, previous work from our laboratory has demonstrated an increase in the severity of IBD and demonstrated increased expression of TNF α , IL-1 β and IL-6 in a mouse line where PPAR γ is specifically deleted throughout the intestinal epithelium, providing evidence for a direct role of PPAR γ in the colon mucosa [1]. However, unexpectedly, the PPAR γ ligand rosiglitazone decreased the severity of DSS-induced colitis and suppressed cytokine production in both colon epithelial-specific PPAR γ null mice and littermate controls, suggesting that PPAR γ expressed in other cell types may also be of importance [1].

Activated macrophages were shown to express high levels of PPAR γ [8], and the PPAR γ agonists, TZDs and 15-deoxy-delta 12,14-prostaglandin J2 were found to suppress the inflammatory response by attenuating expression of specific inflammatory mediators via a PPAR γ -dependent pathway [52]. Macrophages are critical in the pathogenesis of IBD. Depletion of intestinal macrophages was shown to be protective in the IBD mouse models [33,50]. These results suggest that macrophages in the intestine are critical in the pathogenesis of colitis in animal models for IBD. The present study assessed the role of macrophage PPAR γ in DSS-induced IBD. An increased susceptibility to DSS-induced colitis, as defined by body weight loss, diarrhea, rectal bleeding score, colon length and histology, was found in mice that contained a macrophage specific disruption of PPAR γ (PPAR $\gamma^{\Delta M\phi}$) in comparison to wild-type littermate (PPAR $\gamma^{F/F}$) mice. Increased chemokine signaling in colons of PPAR $\gamma^{\Delta M\phi}$ versus PPAR $\gamma^{F/F}$ mice suggest a role for macrophage recruitment in the increased susceptibility of PPAR $\gamma^{\Delta M\phi}$ mice to DSS-induced IBD.

Material and Methods

Animal

PPAR γ -floxed (PPAR $\gamma^{F/F}$) mice containing *loxP* sites flanking exon 2 [2], were crossed with mice harboring the Cre recombinase under control of the lysozyme M promoter (Lys-cre mice). Mice were interbred for over six generations to produce littermates with the same mixed genetic background [10]. Mice, housed in temperature and light controlled rooms, were given water and pelleted chow ad libitum. All animal studies were carried out in accordance with Institute of Laboratory Animal Resources (ILAR) guidelines and approved by the National Cancer Institute Animal Care and Use Committee.

Isolation of macrophage, neutrophils and dendritic cells

Thioglycollate-induced macrophages were isolated from PPAR $\gamma^{\Delta M\phi}$ and PPAR $\gamma^{F/F}$ mice by interperitoneal injection of 3% thioglycollate medium (Becton Dickinson Microbiology Systems, Cockeysville, MD). 72 hours post-injection macrophages were harvested by peritoneal lavage with PBS and plated on plastic non-coated Petri dishes (Becton Dickinson Labware, Franklin Lakes, N.J.) as previously described [2]. Following two hours incubation, plastic-adhered cells were washed three times with PBS and harvested or were further cultured in RPMI media (Invitrogen, Grand Island, NY) containing 2% FBS (Gemini Bio-Products, Woodland, CA) with rosiglitazone (1 μ M) (LKT Laboratories, St. Paul, MN) or vehicle for 24 h. Purity was shown to be above 95% as assessed by F4/80 immunostaining. Neutrophils were

isolated by interperitoneal injection of 3% thioglycollate medium. 4 hours post-injection neutrophils were harvested by peritoneal lavage with PBS and purity was 90% as assessed by Wright Giemsa stain. Dendritic cells were isolated from the spleens of PPAR $\gamma^{\Delta M\phi}$ and PPAR $\gamma^{F/F}$ mice. The spleens were digested with collagenase D and strained through a 100 μ M nylon mesh (BD Biosciences, San Jose, CA) and centrifuged at 400g for 10 minutes. The cell pellet was resuspended in 4 mL of RPMI-1640 media (Invitrogen, Carlsbad, CA) and incubated with Dynabeads (Invitrogen) coated with anti-mouse cd11c antibody (MBL International Corporation, Woburn, MA) for 30 minutes at 4°C with constant agitation. The cell-bound beads were washed five times with RPMI-1640. For cd11c staining, the cells were plated directly on chamber slides (BD Biosciences) and, cd11c was detected using an ABC Mouse Vectastain Elite Kit (Vector Laboratories, Burlingame, CA). The purity was assessed to be greater than 90%. For overnight culture dendritic cells were placed in RPMI-1640 media supplemented with 10% FBS, 2mM L-glutamine, 100 U/mL penicillin/streptomycin, and 50 μ M 2-mercaptoethanol.

Induction and Assessment of colitis

PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice, 8 to 10-weeks-old, were administered 2.5% (wt/vol) DSS (MW, 35,000–44,000) (MP Biomedicals, Aurora, OH) in the drinking water for seven days. Daily changes in body weight and clinical signs of colitis, such as rectal bleeding, diarrhea and bloody stool, were assessed and reported as a score from 0–4. Hemocult SENSE (Beckman Coulter, Inc., Fullerton, CA) was used for the examination of rectal bleeding. For macroscopic colonic damage, colons were opened longitudinally flushed with PBS, and fixed in 10% buffered formalin. The colons were Swiss-rolled to examine entire length of the colon, and processed in paraffin. Colitis was scored on routine hematoxylin and eosin stained section, according to morphological previously described criteria [13].

RNA analysis

RNA was extracted from total colon following DSS administration or from thioglycollate-elicited macrophages using TRIzol reagent (Invitrogen). Northern blot analysis and probes for PPAR γ and acidic ribosomal phosphoprotein (36B4) were previously described [1]. Quantitative real-time PCR (qPCR) was performed using cDNA generated from 1 μ g total RNA with SuperScript III Reverse Transcriptase kit (Invitrogen). Primers were designed for qPCR using the Primer Express software (Applied Biosystems), and sequences are available upon request. qPCR reactions were carried out using SYBR green PCR master mix (Applied Biosystems) in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Values were quantified using the Comparative CT method, and samples normalized to 36B4.

Chemotaxis assay

Transwell inserts with an 8 μ m pore size fitted in 96-well plates (Chemicon International, Temecula, CA) were used for chemotaxis assays. 5×10^5 primary macrophages were loaded into the top well containing 150 μ l of RPMI media. The bottom well contained RPMI media and combination of vehicle, MCP-1 (100ng) (Sigma, St. Louis, MO) or rosiglitazone (1 μ M) as indicated in the figure. The plates were incubated at 37°C in a CO₂ incubator for 4 hours. Following the incubation the migrated cells were detached lysed and fluorescently labeled and analyzed as manufacturer protocol.

Immunohistochemistry

For histological analysis, tissue samples were fixed in 10% neutral buffered formalin overnight and paraffin embedded. 5 μ m sections were cut, deparaffinized with xylene, and hydrated in an ethanol gradient. Immunohistochemical analysis was performed with macrophage receptor with collagenous structure (MARCO) antibody (BD Transduction Laboratories, Lexington,

KY) by a streptavidin-biotin immunoperoxidase method using the ABC Kit (Vector Laboratories). The signal was visualized by diaminobenzidine staining (DAB) (DAKO, Carpinteria, CA) and counterstained with hematoxylin.

Western blot analysis

Thioglycollate-elicited macrophages were isolated from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ and $\text{PPAR}\gamma^{\text{F/F}}$ mice and nuclei were isolated and lysed using NE-PER nuclear extraction kit (Pierce). The macrophage nuclear lysate was prepared for Western blotting as previously described [1]. The membranes were incubated with an antibody against $\text{PPAR}\gamma$ (Santa Cruz Biotechnology Inc, Santa Cruz, CA), and the signals obtained normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Chemicon International).

Data Analysis

Results are expressed as mean $\pm$ S.D. P values were calculated using Independent T Test, or for the DSS experiments multifactorial Anova test on the basis of genotype and DSS status $p < 0.05$ was considered significant.

Results

Generation of Macrophage-specific $\text{PPAR}\gamma$ -null mice

To specifically study the role of macrophage $\text{PPAR}\gamma$ in IBD, $\text{PPAR}\gamma^{\text{F/F}}$ mice were crossed with Lys-Cre transgenic mice to generate mice lacking expression of the $\text{PPAR}\gamma$ in the macrophage. $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice were born at the expected Mendelian frequencies and exhibited no overt abnormalities as compared to $\text{PPAR}\gamma^{\text{F/F}}$ littermate mice. To estimate the extent of macrophage-specific disruption of the $\text{PPAR}\gamma$ gene, PCR analysis was used. The null allele amplifies as a 400 bp product, and was detected in genomic DNA of thioglycollate-elicited macrophages from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice and was not detected in macrophage DNA isolated from $\text{PPAR}\gamma^{\text{F/F}}$ mice (Fig 1A). The intact floxed allele, which amplifies as a 285 bp product was only faintly detected in macrophages from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice. In contrast, the intact floxed allele was the only band evident in macrophages from $\text{PPAR}\gamma^{\text{F/F}}$ mice and from kidney, liver, heart and brown adipose tissue (BAT) genomic DNA from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice (Fig 1A). In addition other myeloid cells were also assessed. In purified spleen dendritic cells (Fig 1A) or whole spleen (data not shown) no recombination was demonstrated. Whereas purified neutrophils demonstrated partial recombination. Consistent with the recombination data, Northern analysis and Western analysis demonstrated that $\text{PPAR}\gamma$ was nearly completely deleted in macrophages from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice (Fig 1B and C). Neutrophils isolated from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ or $\text{PPAR}\gamma^{\text{F/F}}$ mice demonstrated no $\text{PPAR}\gamma$ expression as assessed by qPCR using an exon 2 specific primers consistent with a recent report [28]. To assess the effect of macrophage $\text{PPAR}\gamma$ deletion on gene expression, qPCR was used to analyze mRNAs of $\text{PPAR}\gamma$ target genes. Significant decreases were observed in basal αP2 and CD36 mRNA levels demonstrating a functional consequence of $\text{PPAR}\gamma$ gene disruption in macrophages (Fig 1D).

Cytokine analysis of $\text{PPAR}\gamma$ -null macrophage

To assess whether disruption of $\text{PPAR}\gamma$ in macrophages has any effect on basal cytokine gene expression levels, qPCR was used to measure cytokines mRNA levels shown to be critical in the pathogenesis of IBD. Thioglycollate-elicited macrophage from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ and $\text{PPAR}\gamma^{\text{F/F}}$ mice were incubated with either rosiglitazone ($1\mu\text{M}$) or vehicle for 24 hours. There were no differences in the expression levels of proinflammatory cytokines between macrophages incubated with vehicle from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ versus $\text{PPAR}\gamma^{\text{F/F}}$ mice (Fig. 2). Rosiglitazone only reduced Interleukin (IL)- 1β expression in a $\text{PPAR}\gamma$ -independent manner (Fig. 2). Basal expression of inducible nitric oxide synthase (iNOS) mRNA was increased in

PPAR γ -null macrophages and rosiglitazone decreased basal level of expression only in PPAR $\gamma^{F/F}$, consistent with previous reports [14,35] (Fig. 2). In addition, chemokine signaling was also induced in macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ compared to PPAR $\gamma^{F/F}$ mice. CC chemokine receptor 2 (CCR2) and its ligand monocyte chemoattractant protein-1 (MCP-1) were both increased in PPAR γ -null macrophages (Fig 2), this is the first report demonstrating an increase in MCP-1 expression in PPAR γ -null macrophages. Several studies have demonstrated a repressive role for TZDs in MCP-1 and CCR2 mediated signaling pathways [9,20,45]. Due to concentration specific PPAR γ -independent pathways stimulated by TZDs [52], the present study sought to further characterize the PPAR γ dependency. Rosiglitazone repressed CCR2 and MCP-1 gene expression in macrophages isolated from PPAR $\gamma^{F/F}$ mice (Fig. 2). In contrast, rosiglitazone had no effect on CCR2 and MCP-1 expression in macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ mice (Fig 2), demonstrating that the inhibitory effects of rosiglitazone on CCR2/MCP-1 pathway are PPAR γ dependent.

Susceptibility of PPAR $\gamma^{\Delta M\phi}$ to DSS-induced IBD

PPAR $\gamma^{\Delta M\phi}$ mice (n=21) showed an increased susceptibility to DSS-induced colitis in comparison to PPAR $\gamma^{F/F}$ mice (n=21). Administration of 2.5 % DSS in the drinking water for seven days induced a significant body weight loss in PPAR $\gamma^{\Delta M\phi}$ mice in comparison with PPAR $\gamma^{F/F}$ mice at 5, 6 and 7 days following DSS treatment (Fig. 2A). PPAR $\gamma^{\Delta M\phi}$ mice demonstrated relatively poor diarrhea score and bleeding score (fig 2D, E) following 7-day treatment with DSS, two major clinical symptoms associated with IBD. The colon length of PPAR $\gamma^{\Delta M\phi}$ mice following 7-day treatment with DSS was considerably shortened as compared to PPAR $\gamma^{F/F}$ mice, indicating a greater extent of tissue damage in PPAR $\gamma^{\Delta M\phi}$ (Fig. 3D). Histological analysis showed significantly increased severity of IBD in PPAR $\gamma^{\Delta M\phi}$ mice (fig 3E, F). The colons of PPAR $\gamma^{\Delta M\phi}$ mice following 7-day treatment with DSS demonstrated severe inflammation in the mucosa, muscularis propria, and submucosa with entire loss of the crypts and partial loss of the surface epithelia. The PPAR $\gamma^{\Delta M\phi}$ mice also displayed massive inflammatory infiltrates. Whereas, PPAR $\gamma^{F/F}$ mice displayed only partial loss of the crypts, no loss of the surface epithelia cells and only mild inflammatory infiltrates. These data demonstrate a novel role for macrophage PPAR γ in protection of DSS-induced colonic injury.

Analysis of cytokine expression in DSS-induced IBD

To gain further insight into the mechanism of protection by PPAR γ , cytokine gene expression profiles were compared in whole colons of PPAR $\gamma^{\Delta M\phi}$ and PPAR $\gamma^{F/F}$ mice following 7-day DSS treatment. IFN- γ , IL-1 β , IL-6, IL-10, TNF α , CCR2, MCP-1 and iNOS mRNA were induced from colonic tissue following 7-day treatment of DSS in both PPAR $\gamma^{\Delta M\phi}$ and PPAR $\gamma^{F/F}$ mice (Fig 4). There were no differences in IFN- γ , IL-6, IL-10, and TNF α mRNA expression between PPAR $\gamma^{\Delta M\phi}$ and PPAR $\gamma^{F/F}$ mice. However there was significant increase in IL-1 β , iNOS, CCR2, and MCP-1 expression between colonic RNA isolated from PPAR $\gamma^{\Delta M\phi}$ versus PPAR $\gamma^{F/F}$ mice following 7-day treatment of DSS. Together, these data suggest an important role for macrophage PPAR γ in the regulation of chemokine signaling during increased inflammation.

Role of PPAR γ in monocyte recruitment during DSS-induced IBD

Due to the critical role of chemokine signaling in macrophage recruitment [51], *in-vitro* migration assays were performed on macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ and PPAR $\gamma^{F/F}$ mice. The data demonstrates that basal level of migration is not affected by PPAR γ disruption. However, upon MCP-1 incubation, macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ mice exhibited increased migration when compared to macrophages isolated from PPAR $\gamma^{F/F}$ mice. Furthermore, 1 μ M of rosiglitazone inhibited MCP-1 induced migration in macrophages isolated from PPAR $\gamma^{F/F}$ mice, but had no effect in PPAR γ disrupted macrophages (Fig 5A).

Macrophage specific immunostaining for MARCO was performed on colonic sections following a 7-day treatment with DSS (Fig. 5B). A significant increase in macrophage recruitment was observed in $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice when compared with $\text{PPAR}\gamma^{\text{F/F}}$ mice. To confirm these results, macrophage specific CD68 expression was assessed. Interestingly, CD68 expression was significantly increased in colon tissue of untreated $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice when compared $\text{PPAR}^{\text{F/F}}$, this was not observed by MARCO staining (data not shown), due to the increased sensitivity of qPCR analysis. CD68 expression was enhanced in both $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ and $\text{PPAR}\gamma^{\text{F/F}}$ mice following 7-day treatment with DSS when compared to untreated mice, however the increase in CD68 expression was more pronounced in $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice (Fig. 5C). Taken together, the data demonstrates a novel role for macrophage $\text{PPAR}\gamma$ in the recruitment of macrophages to inflammatory foci in the colon.

Discussion

To examine the role of $\text{PPAR}\gamma$ expressed in macrophages in the mouse inflammatory bowel disease model, macrophage-specific $\text{PPAR}\gamma$ -null mice were generated using the Cre/loxP strategy with the Lys-cre transgene. $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice exhibited over 90% loss of $\text{PPAR}\gamma$ expression in macrophage as assessed by Northern and Western blot analysis. In a DSS-induced IBD model, $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice exhibited more severe colitis as revealed by loss of body weight, shortened colon length, diarrhea, rectal bleeding scores, and macroscopic and histological analysis in comparison with $\text{PPAR}\gamma^{\text{F/F}}$ mice. Cytokine gene expression profiles were compared in macrophages and colonic tissue from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ and $\text{PPAR}\gamma^{\text{F/F}}$ mice. iNOS, MCP-1 and CCR2 mRNA levels were significantly increased in macrophage from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice. Similarly, in colonic tissue following 7-day treatment with DSS, the mRNAs encoding the chemotactic proteins, MCP-1 and CCR2, and iNOS, were induced. In addition there was a significant increase in IL-1 β cytokine gene expression in $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ versus $\text{PPAR}\gamma^{\text{F/F}}$ mice. This is consistent with an anti-inflammatory role for $\text{PPAR}\gamma$ in macrophage, and provides further support for a critical role for macrophages in colitis. from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice demonstrated an increased expression of iNOS, MCP-1 and CCR2 when compared to macrophages isolated from $\text{PPAR}\gamma^{\text{F/F}}$ mice. Therefore the data suggest through endogenous $\text{PPAR}\gamma$ ligands

Increasing evidence suggests that the MCP-1/CCR2 pathway is important in inflammatory diseases. In an atherosclerotic mouse model, inhibition of CCR2 or its ligand MCP-1, significantly decreased macrophage recruitment and lesion size [7,15,19]. In addition, a functional polymorphism of the CCR2 gene locus, which reduces CCR2 activity, is associated with decreased risk of coronary atherosclerosis. [48] Recently, it was demonstrated that $\text{PPAR}\gamma$ disruption in macrophage increases CCR2 expression and recruitment of macrophages to atherogenic sites. [4] In the DSS-induced IBD model, a novel chemokine receptor antagonist TAK-779 that demonstrates high affinity for CCR2, protected mice following DSS administration. The ameliorative effects of TAK-779 were directly correlated with a decrease in macrophage recruitment into the colonic mucosa [46]. In addition, specific disruption of CCR5 and/or CCR2 protected mice from the severe inflammation and mucosal damage induced by DSS. Interestingly, the disruption of CCR2 and CCR5 was not critical for macrophage migration. Instead, both chemokine receptors were able to regulate the migration and differentiation of mucosal T cells [3]. This discrepancy may be due to differences in MCP-1 expression levels. MCP-1 levels were not altered in mice with selective deletion of CCR2 or CCR5 [3]. Consistent with an earlier report [46], the present study demonstrates increased CCR2 and MCP-1 levels in inflamed colonic tissue from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice, which correlates with an increase in macrophage recruitment to the colon.

In addition to chemokine signaling, IL-1 β and iNOS expression were increased in colons from $\text{PPAR}\gamma^{\Delta\text{M}\phi}$ mice when compared to $\text{PPAR}\gamma^{\text{F/F}}$ mice. IL-1 β , a pro-inflammatory cytokine was

shown to be critical in gut inflammation by activating numerous immune cell types and appears to be a primary cause of IBD-induced diarrhea [18,39]. IL-1 β at high doses induces tissue damage by promoting epithelial cell necrosis, and inhibiting the endogenous action of IL-1 β ameliorates acute and chronic experimental colitis [11,12,39].

iNOS expression is regulated by several pro-inflammatory cytokines, and it functions to generate high levels of nitric oxide (NO) via oxidative metabolism of L-arginine. NO activates guanylate cyclases enhancing cyclic GMP synthesis and is involved in various functions including vasodilatation, inhibition of platelet aggregation, skeletal muscle contractility, and host defense. [43] In addition, NO is a highly reactive free radical and can rapidly react with active oxygen species to generate peroxynitrite and cause severe detrimental effects [36,41]. However, IBD models using partially selective iNOS inhibitors or iNOS deficient mice, has led to contradictory results [6,16,22,29,32,49,53]. The dual nature of NO is thought to be the reason for conflicting data in determining the role of iNOS in IBD models. The present data demonstrates that local increases in iNOS expression correlate with increased severity of IBD.

Unstimulated macrophages from PPAR $\gamma^{\Delta M\phi}$ mice displayed an increase in several proinflammatory mediators. In addition colons from PPAR $\gamma^{\Delta M\phi}$ mice displayed increase in macrophage recruitment as assessed by CD68 expression. The present study demonstrates a critical role of endogenous PPAR γ ligands in maintaining homeostasis in respect to inflammatory responses. Therefore, the data suggest in the absence of macrophage PPAR γ the anti-inflammatory signal from endogenous PPAR γ ligands are no longer conveyed, making them more susceptible to inflammatory diseases. While the expression levels of a number of proinflammatory genes are induced in PPAR $\gamma^{\Delta M\phi}$ mice following administration of DSS, the relative importance of these and possibly other genes in the increased susceptibility of PPAR $\gamma^{\Delta M\phi}$ mice in DSS-induced IBD model remains unclear. However, the present study has identified a novel and critical role for macrophage PPAR γ in the recruitment and activation of macrophages in the pathogenesis of DSS-induced colitis. These events appear to represent a deleterious cyclical process in which recruited macrophages secrete proinflammatory cytokines that in turn recruit additional macrophages, and ultimately cause severe tissue damage. The present study provides rationale to target macrophage PPAR γ in patients diagnosed with IBD. Recently, it was shown that the molecular mechanisms driving ligand-dependent trans-repression by PPAR γ in macrophage are distinct from classical PPAR γ -dependent gene transcription [35]. It will be of interest to explore how these mechanisms can be used to develop new treatment modalities for IBD.

Acknowledgements

This study was supported by the National Cancer Institute Intramural Research Program and Y.M.S was supported by a postdoctoral fellowship from the American Cancer Society PF-06-014-01-CNE.

References

1. Adachi M, Kurotani R, Morimura K, et al. PPAR{gamma} in colonic epithelial cells protects against experimental inflammatory bowel disease. *Gut*. 2006
2. Akiyama TE, Sakai S, Lambert G, et al. Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. *Mol Cell Biol* 2002;22:2607–19. [PubMed: 11909955]
3. Andres PG, Beck PL, Mizoguchi E, et al. Mice with a selective deletion of the CC chemokine receptors 5 or 2 are protected from dextran sodium sulfate-mediated colitis: lack of CC chemokine receptor 5 expression results in a NK1.1+ lymphocyte-associated Th2-type immune response in the intestine. *J Immunol* 2000;164:6303–12. [PubMed: 10843684]
4. Babaev VR, Yancey PG, Ryzhov SV, et al. Conditional knockout of macrophage PPARgamma increases atherosclerosis in C57BL/6 and low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2005;25:1647–53. [PubMed: 15947238]

5. Bassaganya-Riera J, Reynolds K, Martino-Catt S, et al. Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenterology* 2004;127:777–91. [PubMed: 15362034]
6. Blanchard HS, Dernis-Labous E, Lamarque D, et al. Inducible nitric oxide synthase attenuates chronic colitis in human histocompatibility antigen HLA-B27/human beta2 microglobulin transgenic rats. *Eur Cytokine Netw* 2001;12:111–8. [PubMed: 11282554]
7. Boring L, Gosling J, Cleary M, et al. Decreased lesion formation in CCR2^{-/-} mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature* 1998;394:894–7. [PubMed: 9732872]
8. Chawla A, Barak Y, Nagy L, et al. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat Med* 2001;7:48–52. [PubMed: 11135615]
9. Chen Y, Green SR, Ho J, et al. The mouse CCR2 gene is regulated by two promoters that are responsive to plasma cholesterol and peroxisome proliferator-activated receptor gamma ligands. *Biochem Biophys Res Commun* 2005;332:188–93. [PubMed: 15896316]
10. Clausen BE, Burkhardt C, Reith W, et al. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res* 1999;8:265–77. [PubMed: 10621974]
11. Cominelli F, Nast CC, Clark BD, et al. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. *J Clin Invest* 1990;86:972–80. [PubMed: 2168444]
12. Cominelli F, Nast CC, Duchini A, et al. Recombinant interleukin-1 receptor antagonist blocks the proinflammatory activity of endogenous interleukin-1 in rabbit immune colitis. *Gastroenterology* 1992;103:65–71. [PubMed: 1535326]
13. Cooper HS, Murthy SN, Shah RS, et al. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 1993;69:238–49. [PubMed: 8350599]
14. Crosby MB, Svenson J, Gilkeson GS, et al. A novel PPAR response element in the murine iNOS promoter. *Mol Immunol* 2005;42:1303–10. [PubMed: 15950726]
15. Dawson TC, Kuziel WA, Osahar TA, et al. Absence of CC chemokine receptor-2 reduces atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* 1999;143:205–11. [PubMed: 10208497]
16. Dikopoulos N, Nussler AK, Liptay S, et al. Inhibition of nitric oxide synthesis by aminoguanidine increases intestinal damage in the acute phase of rat TNB-colitis. *Eur J Clin Invest* 2001;31:234–9. [PubMed: 11264651]
17. Dubuquoy L, Jansson EA, Deeb S, et al. Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology* 2003;124:1265–76. [PubMed: 12730867]
18. Elson CO, Sartor RB, Tennyson GS, et al. Experimental models of inflammatory bowel disease. *Gastroenterology* 1995;109:1344–67. [PubMed: 7557106]
19. Gosling J, Slaymaker S, Gu L, et al. MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. *J Clin Invest* 1999;103:773–8. [PubMed: 10079097]
20. Han KH, Chang MK, Boullier A, et al. Oxidized LDL reduces monocyte CCR2 expression through pathways involving peroxisome proliferator-activated receptor gamma. *J Clin Invest* 2000;106:793–802. [PubMed: 10995790]
21. He W, Barak Y, Hevener A, et al. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 2003;100:15712–7. [PubMed: 14660788]
22. Hokari R, Kato S, Matsuzaki K, et al. Reduced sensitivity of inducible nitric oxide synthase-deficient mice to chronic colitis. *Free Radic Biol Med* 2001;31:153–63. [PubMed: 11440827]
23. Imai T, Takakuwa R, Marchand S, et al. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proc Natl Acad Sci U S A* 2004;101:4543–7. [PubMed: 15070754]
24. Jones JR, Barrick C, Kim KA, et al. Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc Natl Acad Sci U S A* 2005;102:6207–12. [PubMed: 15833818]
25. Katayama K, Wada K, Nakajima A, et al. A novel PPAR gamma gene therapy to control inflammation associated with inflammatory bowel disease in a murine model. *Gastroenterology* 2003;124:1315–24. [PubMed: 12730872]

26. Kliewer SA, Lenhard JM, Willson TM, et al. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* 1995;83:813–9. [PubMed: 8521498]
27. Kliewer SA, Sundseth SS, Jones SA, et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A* 1997;94:4318–23. [PubMed: 9113987]
28. Kobayashi M, Thomassen MJ, Rambasek T, et al. An inverse relationship between peroxisome proliferator-activated receptor gamma and allergic airway inflammation in an allergen challenge model. *Ann Allergy Asthma Immunol* 2005;95:468–73. [PubMed: 16312170]
29. Kriegelstein CF, Cerwinka WH, Laroux FS, et al. Regulation of murine intestinal inflammation by reactive metabolites of oxygen and nitrogen: divergent roles of superoxide and nitric oxide. *J Exp Med* 2001;194:1207–18. [PubMed: 11696587]
30. Lehmann JM, Moore LB, Smith-Oliver TA, et al. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 1995;270:12953–6. [PubMed: 7768881]
31. Lytle C, Tod TJ, Vo KT, et al. The peroxisome proliferator-activated receptor gamma ligand rosiglitazone delays the onset of inflammatory bowel disease in mice with interleukin 10 deficiency. *Inflamm Bowel Dis* 2005;11:231–43. [PubMed: 15735429]
32. Nakamura H, Tsukada H, Oya M, et al. Aminoguanidine has both an anti-inflammatory effect on experimental colitis and a proliferative effect on colonic mucosal cells. *Scand J Gastroenterol* 1999;34:1117–22. [PubMed: 10582763]
33. Nakase H, Okazaki K, Tabata Y, et al. Biodegradable microspheres targeting mucosal immune-regulating cells: new approach for treatment of inflammatory bowel disease. *J Gastroenterol* 2003;38 (Suppl 15):59–62. [PubMed: 12698874]
34. Olefsky JM. Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists. *J Clin Invest* 2000;106:467–72. [PubMed: 10953021]
35. Pascual G, Fong AL, Ogawa S, et al. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* 2005;437:759–63. [PubMed: 16127449]
36. Pryor WA, Squadrito GL. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 1995;268:L699–722. [PubMed: 7762673]
37. Rosen ED, Sarraf P, Troy AE, et al. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell* 1999;4:611–7. [PubMed: 10549292]
38. Sanchez-Hidalgo M, Martin AR, Villegas I, et al. Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, reduces chronic colonic inflammation in rats. *Biochem Pharmacol* 2005;69:1733–44. [PubMed: 15876425]
39. Sartor RB. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology* 1994;106:533–9. [PubMed: 8299918]
40. Spiegelman BM, Hu E, Kim JB, et al. PPAR gamma and the control of adipogenesis. *Biochimie* 1997;79:111–2. [PubMed: 9209705]
41. Squadrito GL, Pryor WA. The formation of peroxynitrite in vivo from nitric oxide and superoxide. *Chem Biol Interact* 1995;96:203–6. [PubMed: 7728908]
42. Su CG, Wen X, Bailey ST, et al. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. *J Clin Invest* 1999;104:383–9. [PubMed: 10449430]
43. Suschek CV, Schnorr O, Kolb-Bachofen V. The role of iNOS in chronic inflammatory processes in vivo: is it damage-promoting, protective, or active at all? *Curr Mol Med* 2004;4:763–75. [PubMed: 15579023]
44. Takagi T, Naito Y, Tomatsuri N, et al. Pioglitazone, a PPAR-gamma ligand, provides protection from dextran sulfate sodium-induced colitis in mice in association with inhibition of the NF-kappaB-cytokine cascade. *Redox Rep* 2002;7:283–9. [PubMed: 12688511]
45. Tanaka T, Fukunaga Y, Itoh H, et al. Therapeutic potential of thiazolidinediones in activation of peroxisome proliferator-activated receptor gamma for monocyte recruitment and endothelial regeneration. *Eur J Pharmacol* 2005;508:255–65. [PubMed: 15680279]

46. Tokuyama H, Ueha S, Kurachi M, et al. The simultaneous blockade of chemokine receptors CCR2, CCR5 and CXCR3 by a non-peptide chemokine receptor antagonist protects mice from dextran sodium sulfate-mediated colitis. *Int Immunol* 2005;17:1023–34. [PubMed: 16000328]
47. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* 1994;63:451–86. [PubMed: 7979245]
48. Valdes AM, Wolfe ML, O'Brien EJ, et al. Val64Ile polymorphism in the C-C chemokine receptor 2 is associated with reduced coronary artery calcification. *Arterioscler Thromb Vasc Biol* 2002;22:1924–8. [PubMed: 12426226]
49. Vallance BA, Dijkstra G, Qiu B, et al. Relative contributions of NOS isoforms during experimental colitis: endothelial-derived NOS maintains mucosal integrity. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G865–74. [PubMed: 15217783]
50. Watanabe N, Ikuta K, Okazaki K, et al. Elimination of local macrophages in intestine prevents chronic colitis in interleukin-10-deficient mice. *Dig Dis Sci* 2003;48:408–14. [PubMed: 12643623]
51. Weber C, Schober A, Zernecke A. Chemokines: key regulators of mononuclear cell recruitment in atherosclerotic vascular disease. *Arterioscler Thromb Vasc Biol* 2004;24:1997–2008. [PubMed: 15319268]
52. Welch JS, Ricote M, Akiyama TE, et al. PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. *Proc Natl Acad Sci U S A* 2003;100:6712–7. [PubMed: 12740443]
53. Zingarelli B, Cuzzocrea S, Szabo C, et al. Mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. *J Pharmacol Exp Ther* 1998;287:1048–55. [PubMed: 9864291]

Figure 1. Macrophage-specific disruption of PPAR γ

(A) PCR analysis of the recombination of PPAR γ allele in macrophage (Mφ), dendritic cells (DC) or neutrophil (neut) genomic DNA isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice or from heart, kidney, liver or brown adipose tissue (BAT) from PPAR $\gamma^{\Delta M\phi}$ mice. (B) Northern blot analysis measuring PPAR γ expression in total RNA from macrophage cells isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice. Expression was normalized to 36B4 gene expression. (C) Western blot analysis measuring PPAR γ expression in 10μg nuclear lysate from macrophage cells isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice. Expression was normalized to GAPDH protein expression, and CV-1 cells transfected with PPAR γ served as positive control. (D) qPCR analysis of cd36 and aP2 mRNA in macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice. Expression was normalized to 36B4 and each bar represents the mean value $\pm$ S.D. (*) = $P < .05$ compared to macrophage isolated from PPAR $\gamma^{F/F}$ mice.

Figure 2. Cytokine expression in macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice
 iNOS, CCR2, IL-1 β , IL-10, IL-6, TNF α , MCP-1 and IFN γ mRNA expression was assessed by qPCR in macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ incubated with vehicle (Veh) (black bars) or 1 μ M rosiglitazone (Rosi) for 24 hours (open bars). Expression was normalized to 36B4 and each bar represents the mean value $\pm$ S.D. (*) = $p < 0.05$ compared to vehicle incubated macrophages isolated from PPAR $\gamma^{F/F}$ mice. (†) = $p < 0.05$ compared to vehicle incubated macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ mice.

Figure 3. Clinical assessment of DSS-induced IBD in $PPAR_{\gamma}^{\Delta M\phi}$ or $PPAR_{\gamma}^{F/F}$ mice
 (A) Body weight changes following DSS-induction of colitis, (B) colon length, (C) diarrhea score, (D) bleeding score, (E) representative H & E stained colon sections (F) and histology score. Data represent the mean value $\pm$ S.D of $n=21$ $PPAR_{\gamma}^{\Delta M\phi}$ and $n=21$ $PPAR_{\gamma}^{F/F}$ mice, (*) = $p < 0.05$ compared to $PPAR_{\gamma}^{F/F}$ DSS treated mice.

Figure 4. Cytokine expression from colonic tissue following seven-day DSS or control treatment from $PPAR\gamma^{\Delta M\phi}$ or $PPAR\gamma^{F/F}$ mice
 iNOS, CCR2, IL-1 β , IL-10, IL-6, TNF α , MCP-1 and IFN γ mRNA expression was assessed by qPCR in from colonic tissue in $PPAR\gamma^{\Delta M\phi}$ or $PPAR\gamma^{F/F}$ mice given normal drinking water (Con) or water containing 2.5% DSS for seven days (DSS). Expression was normalized to 36B4 and each bar represents the mean value $\pm$ S.D. (*) = $p < 0.05$ compared to colons from $PPAR\gamma^{F/F}$ mice following 7-day DSS treatment.

Figure 5. Chemotactic response of macrophages isolated from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice
 (A) In-vitro migration activity of macrophages incubated with vehicle (Veh), 100ng MCP-1 (Tam) or 1 μ M rosiglitazone (Rosi) or co-incubated with MCP-1 and Rosi for 4 hours each bar represents the mean value $\pm$ S.D. (*) = $p < 0.05$ compared to macrophages incubated with MCP-1 from PPAR $\gamma^{F/F}$ mice. (B) Immunostaining of MARCO in colonic tissue following 7 day DSS treatment from PPAR $\gamma^{\Delta M\phi}$ or PPAR $\gamma^{F/F}$ mice. Inset is an increased magnification. (C) Macrophage specific CD68 marker assessed by qPCR. Expression normalized to 36B4 and each bar represents the mean value $\pm$ S.D. (†) = $p < 0.05$ compared to PPAR $\gamma^{F/F}$ mice given normal drinking water (Con). (*) = $p < 0.05$ compared to PPAR $\gamma^{F/F}$ given water containing 2.5% DSS.