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

Yatrik M Shah[1], **Keiichirou Morimura**[1], and **Frank J Gonzalez**[1,2]
1*Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD*

Abstract

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

Methods—Mice with a targeted disruption of PPARγ in macrophages ($PPAR\gamma^{\Delta M\varphi}$) 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—$PPAR\gamma^{\Delta M\varphi}$ 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)-1β, CCR2, MCP-1 and iNOS mRNA levels in colons of $PPAR\gamma^{\Delta M\varphi}$ 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 PPARγ in the DSS-induced IBD model. The data suggest that PPARγ 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 (PPARγ) is a member of the nuclear receptor superfamily of transcription factors. Following activation of PPARγ 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], PPARγ 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 PPARγ and are in widespread clinical use for anti-type 2 diabetes therapy. [34] PPARγ is highly expressed in brown and white adipose tissue,

[2]Correspondence 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: fjgonz@helix.nih.gov.

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

More recently, PPARγ 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. PPARγ 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 TNFα, IL-1β and IL-6 in a mouse line where PPARγ is specifically deleted throughout the intestinal epithelium, providing evidence for a direct role of PPARγ in the colon mucosa [1]. However, unexpectedly, the PPARγ ligand rosiglitazone decreased the severity of DSS-induced colitis and suppressed cytokine production in both colon epithelial-specific PPARγ null mice and littermate controls, suggesting that PPARγ expressed in other cell types may also be of importance [1].

Activated macrophages were shown to express high levels of PPARγ [8], and the PPARγ 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 PPARγ-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 PPARγ 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 PPARγ ($PPAR\gamma^{\Delta M\varphi}$) in comparison to wild-type littermate ($PPAR\gamma^{F/F}$) mice. Increased chemokine signaling in colons of $PPAR\gamma^{\Delta M\varphi}$ versus $PPAR\gamma^{F/F}$ mice suggest a role for macrophage recruitment in the increased susceptibility of $PPAR\gamma^{\Delta M\varphi}$ mice to DSS-induced IBD.

Material and Methods

Animal

PPARγ-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 $PPAR\gamma^{\Delta M\varphi}$ and $PPAR\gamma^{F/F}$ mice by interperitoneal injection of 3% thioglycollate medium (Becton Dickinson Microbiology Systems, Cockeyesville, 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 (1μM) (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$\gamma^{\Delta M\varphi}$ and PPAR$\gamma^{F/F}$ mice. The spleens were digested with collagenase D and strained through a 100μM nylon mesh (BD Biosciences, San Jose, CA) and centrifuged at 400*g* 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 50μM 2-mercaptoethanol.

Induction and Assessment of colitis

PPAR$\gamma^{\Delta M\varphi}$ 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. Hemoccult SENSA (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 PPARγ and acidic ribosomal phosphoprotein (36B4) were previously described [1]. Quantitative real-time PCR (qPCR) was performed using cDNA generated from 1μg 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 8μm 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 150μl of RPMI media. The bottom well contained RPMI media and combination of vehicle, MCP-1 (100ng) (Sigma, St. Louis, MO) or rosiglitazone (1μM) as indicated in the figure. The plates were incubated at 37°C in a CO_2 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. 5μm 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 PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ 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 PPARγ (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 ± 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 PPARγ-null mice

To specifically study the role of macrophage PPARγ in IBD, PPARγ$^{F/F}$ mice were crossed with Lys-Cre transgenic mice to generate mice lacking expression of the PPARγ in the macrophage. PPARγ$^{\Delta M\varphi}$ mice were born at the expected Mendelian frequencies and exhibited no overt abnormalities as compared to PPARγ$^{F/F}$ littermate mice. To estimate the extent of macrophage-specific disruption of the PPARγ 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 PPARγ$^{\Delta M\varphi}$ mice and was not detected in macrophage DNA isolated from PPARγ$^{F/F}$ mice (Fig 1A). The intact floxed allele, which amplifies as a 285 bp product was only faintly detected in macrophages from PPARγ$^{\Delta M\varphi}$ mice. In contrast, the intact floxed allele was the only band evident in macrophages from PPARγ$^{F/F}$ mice and from kidney, liver, heart and brown adipose tissue (BAT) genomic DNA from PPARγ$^{\Delta M\varphi}$ 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 PPARγ was nearly completely deleted in macrophages from PPARγ$^{\Delta M\varphi}$ mice (Fig 1B and C). Neutrophils isolated from PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ mice demonstrated no PPARγ expression as assessed by qPCR using an exon 2 specific primers consistent with a recent report [28]. To assess the effect of macrophage PPARγ deletion on gene expression, qPCR was used to analyze mRNAs of PPARγ target genes. Significant decreases were observed in basal aP2 and CD36 mRNA levels demonstrating a functional consequence of PPARγ gene disruption in macrophages (Fig 1D).

Cytokine analysis of PPARγ-null macrophage

To assess whether disruption of PPARγ in macrophages has any affect 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 PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ mice were incubated with either rosiglitazone (1μM) or vehicle for 24 hours. There were no differences in the expression levels of proinflammatory cytokines between macrophages incubated with vehicle from PPARγ$^{\Delta M\varphi}$ versus PPARγ$^{F/F}$ mice (Fig. 2). Rosiglitazone only reduced Interleukin (IL)-1β expression in a PPARγ-independent manner (Fig. 2). Basal expression of inducible nitric oxide synthase (iNOS) mRNA was increased in

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

Susceptibility of PPARγ$^{\Delta M\varphi}$ to DSS-induced IBD

PPARγ$^{\Delta M\varphi}$ mice (n=21) showed an increased susceptibility to DSS-induced colitis in comparison to PPARγ$^{F/F}$ mice (n=21). Administration of 2.5 % DSS in the drinking water for seven days induced a significant body weight loss in PPARγ$^{\Delta M\varphi}$ mice in comparison with PPARγ$^{F/F}$ mice at 5, 6 and 7 days following DSS treatment (Fig. 2A). PPARγ$^{\Delta M\varphi}$ 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 PPARγ$^{\Delta M\varphi}$ mice following 7-day treatment with DSS was considerably shortened as compared to PPARγ$^{F/F}$ mice, indicating a greater extent of tissue damage in PPARγ$^{\Delta M\varphi}$ (Fig. 3D). Histological analysis showed significantly increased severity of IBD in PPARγ$^{\Delta M\varphi}$ mice (fig 3E, F). The colons of PPARγ$^{\Delta M\varphi}$ 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 PPARγ$^{\Delta M\varphi}$ mice also displayed massive inflammatory infiltrates. Whereas, PPARγ$^{F/F}$ 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 PPARγ 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 PPARγ, cytokine gene expression profiles were compared in whole colons of PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ mice following 7-day DSS treatment. IFN-γ, IL-1β, IL-6, IL-10, TNFα, CCR2, MCP-1 and iNOS mRNA were induced from colonic tissue following 7-day treatment of DSS in both PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ mice (Fig 4). There were no differences in IFN-γ, IL-6, IL-10, and TNFα mRNA expression between PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ mice. However there was significant increase in IL-1β, iNOS, CCR2, and MCP-1 expression between colonic RNA isolated from PPARγ$^{\Delta M\varphi}$ versus PPARγ$^{F/F}$ mice following 7-day treatment of DSS. Together, these data suggest an important role for macrophage PPARγ in the regulation of chemokine signaling during increased inflammation.

Role of PPARγ 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 PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ mice. The data demonstrates that basal level of migration is not affected by PPARγ disruption. However, upon MCP-1 incubation, macrophages isolated from PPARγ$^{\Delta M\varphi}$ mice exhibited increased migration when compared to macrophages isolated from PPARγ$^{F/F}$ mice. Furthermore, 1μM of rosiglitazone inhibited MCP-1 induced migration in macrophages isolated from PPARγ$^{F/F}$ mice, but had no effect in PPARγ 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 PPARγ$^{\Delta M\varphi}$ mice when compared with PPARγ$^{F/F}$ mice. To confirm these results, macrophage specific CD68 expression was assessed. Interestingly, CD68 expression was significantly increased in colon tissue of untreated PPARγ$^{\Delta M\varphi}$ mice when compared PPAR$^{F/F}$, this was not observed by MARCO staining (data not shown), due to the increased sensitivity of qPCR analysis. CD68 expression was enhanced in both PPARγ$^{\Delta M\varphi}$ and PPARγ$^{F/F}$ mice following 7-day treatment with DSS when compared to untreated mice, however the increase in CD68 expression was more pronounced in PPARγ$^{\Delta M\varphi}$ mice (Fig. 5C). Taken together, the data demonstrates a novel role for macrophage PPARγ in the recruitment of macrophages to inflammatory foci in the colon.

Discussion

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

In addition to chemokine signaling, IL-1β and iNOS expression were increased in colons from PPARγ$^{\Delta M\varphi}$ mice when compared to PPARγ$^{F/F}$ mice. IL-1β, 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-1β at high doses induces tissue damage by promoting epithelial cell necrosis, and inhibiting the endogenous action of IL-1β 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 PPARγ$^{\Delta M\varphi}$ mice displayed an increase in several proinflammatory mediators. In addition colons from PPARγ$^{\Delta M\varphi}$ mice displayed increase in macrophage recruitment as assessed by CD68 expression. The present study demonstrates a critical role of endogenous PPARγ ligands in maintaining homeostasis in respect to inflammatory responses. Therefore, the data suggest in the absence of macrophage PPARγ the anti-inflammatory signal from endogenous PPARγ 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 PPARγ$^{\Delta M\varphi}$ mice following administration of DSS, the relative importance of these and possibly other genes in the increased susceptibility of PPARγ$^{\Delta M\varphi}$ mice in DSS-induced IBD model remains unclear. However, the present study has identified a novel and critical role for macrophage PPARγ 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 PPARγ in patients diagnosed with IBD. Recently, it was shown that the molecular mechanisms driving ligand-dependent trans-repression by PPARγ in macrophage are distinct from classical PPARγ-dependent gene transcription [35]. It will be of interest to explore how these mechanisms can be used to develop new treatment modalities for IBD.

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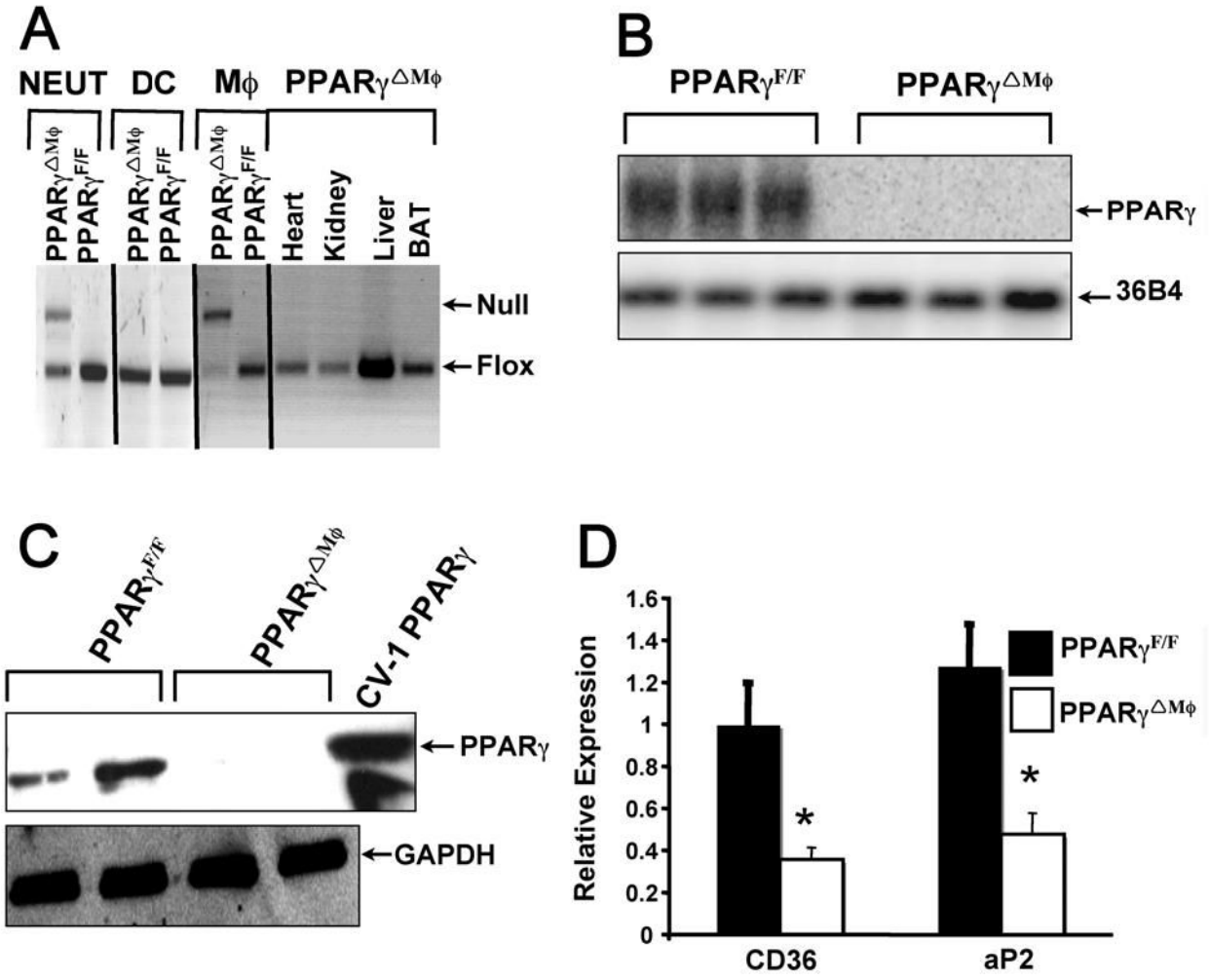

Figure 1. Macrophage-specific disruption of PPARγ

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

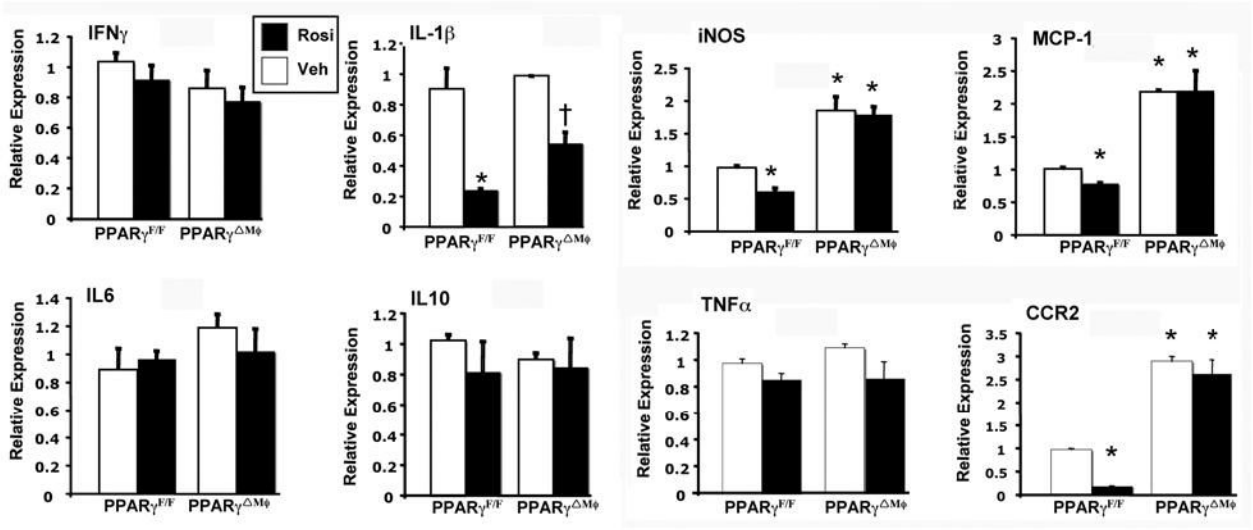

Figure 2. Cytokine expression in macrophages isolated from PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ mice

iNOS, CCR2, IL-1β, Il-10, IL-6, TNFα, MCP-1 and IFNγ mRNA expression was assessed by qPCR in macrophages isolated from PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ incubated with vehicle (Veh) (black bars) or 1 μM rosiglitazone (Rosi) for 24 hours (open bars). Expression was normalized to 36B4 and each bar represents the mean value ± S.D. (*)= $p < 0.05$ compared to vehicle incubated macrophages isolated from PPARγ$^{F/F}$ mice. (†)= $p < 0.05$ compared to vehicle incubated macrophages isolated from PPARγ$^{\Delta M\varphi}$ mice.

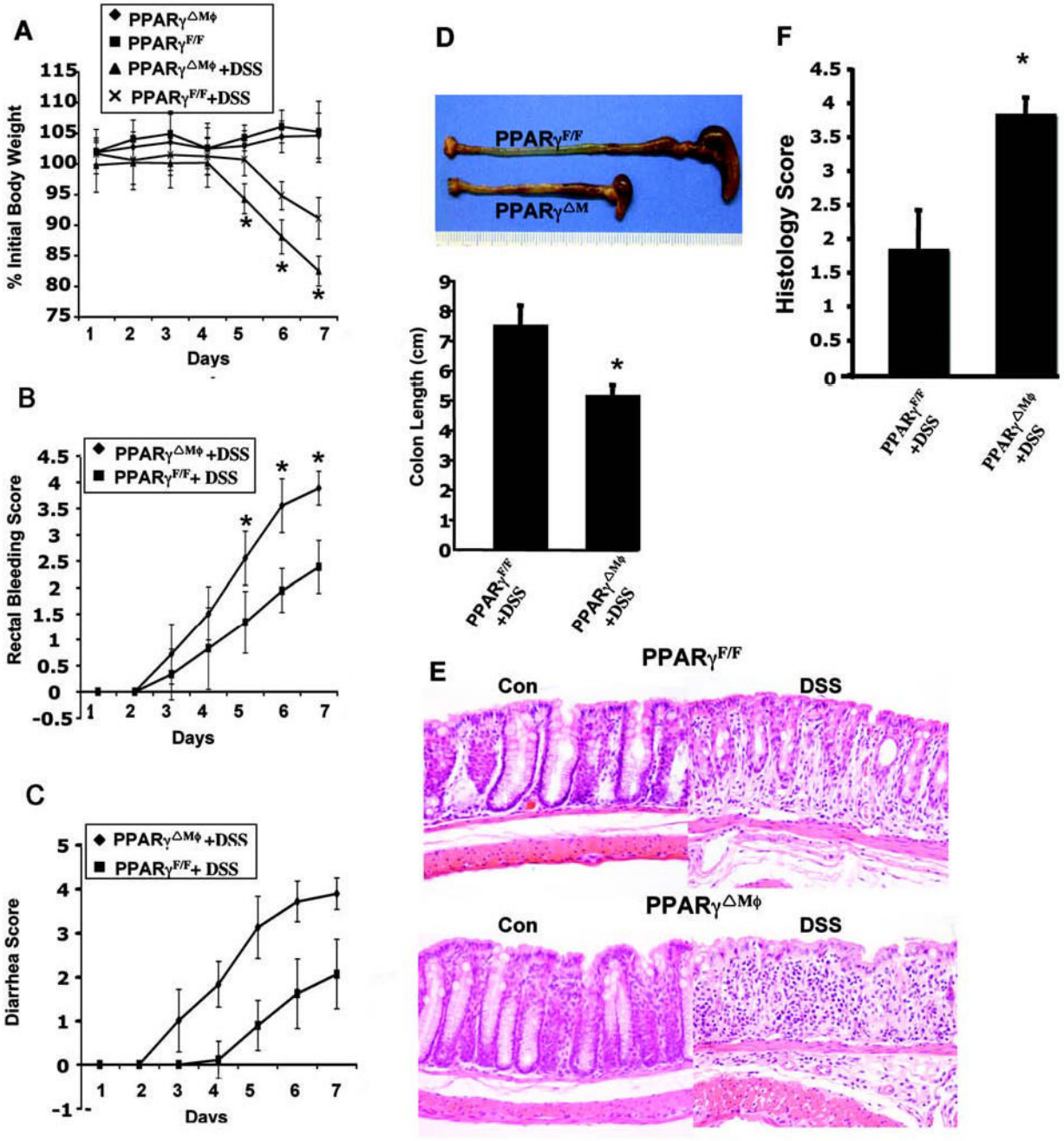

Figure 3. Clinical assessment of DSS-induced IBD in PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ mice

(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 ± S.D of n=21 PPARγ$^{\Delta M\varphi}$ and n=21 PPARγ$^{F/F}$ mice, (*) = $p < 0.05$ compared to PPARγ$^{F/F}$ DSS treated mice.

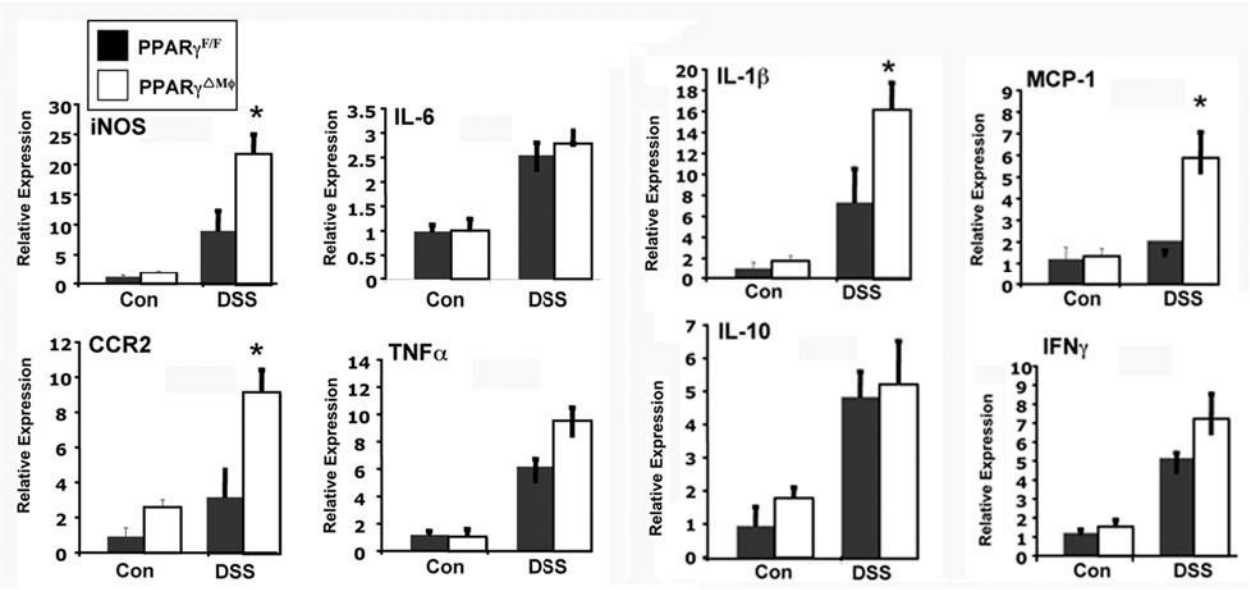

Figure 4. Cytokine expression from colonic tissue following seven-day DSS or control treatment from PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ mice

iNOS, CCR2, IL-1β, Il-10, IL-6, TNFα, MCP-1 and IFNγ mRNA expression was assessed by qPCR in from colonic tissue in PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ 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 ± S.D. (*)= $p < 0.05$ compared to colons from PPARγ$^{F/F}$ mice following 7-day DSS treatment.

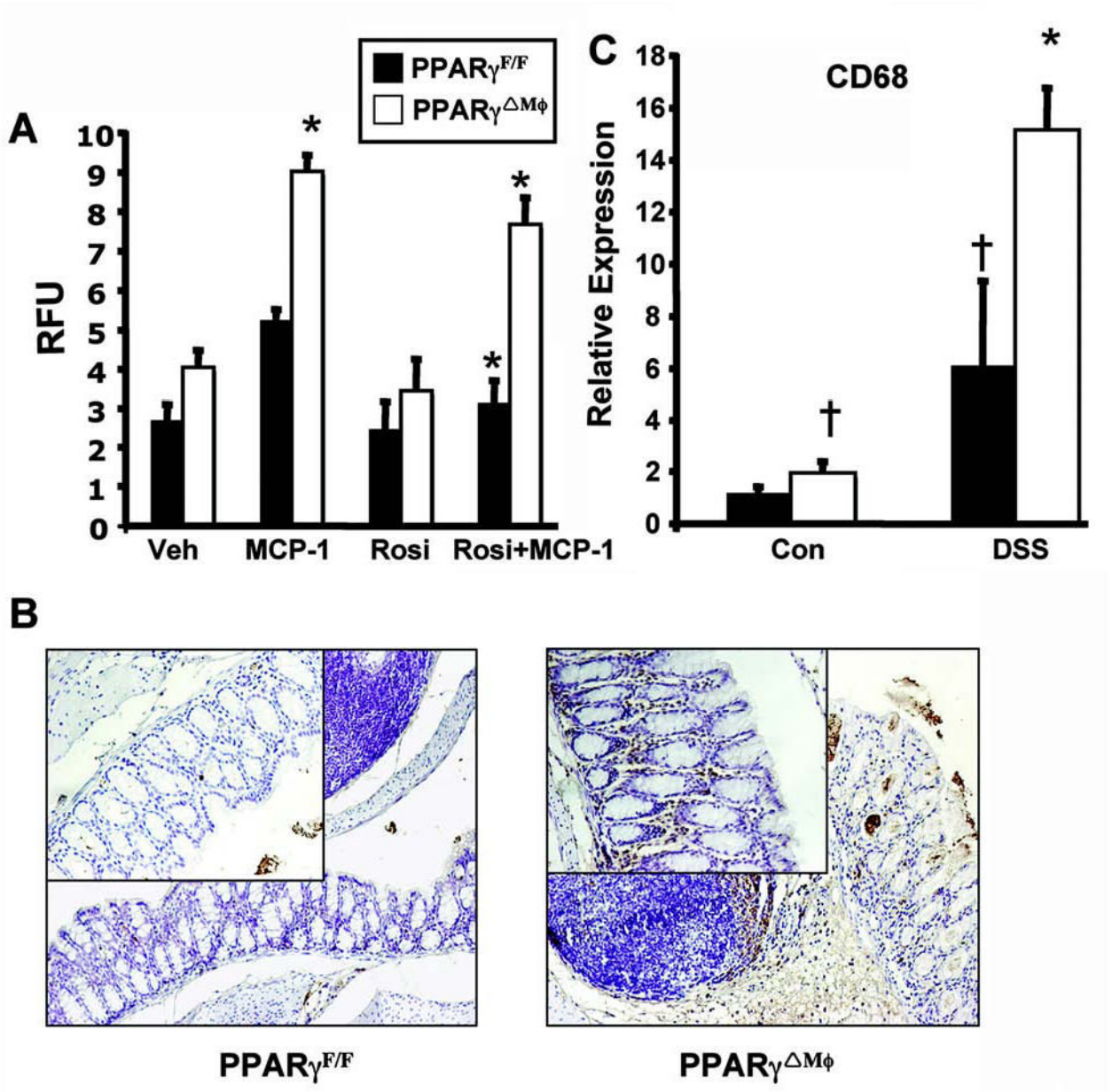

Figure 5. Chemotatic response of macrophages isolated from PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ mice

(A) In-vitro migration activity of macrophages incubated with vehicle (Veh), 100ng MCP-1 (Tam) or 1μM rosiglitazone (Rosi) or co-incubated with MCP-1 and Rosi for 4 hours each bar represents the mean value ± S.D. (*)= $p < 0.05$ compared to macrophages incubated with MCP-1 from PPARγ$^{F/F}$ mice. (B) Immunostaining of MARCO in colonic tissue following 7 day DSS treatment from PPARγ$^{\Delta M\varphi}$ or PPARγ$^{F/F}$ 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 ± S.D. (†)= $p < 0.05$ compared to PPARγ$^{F/F}$ mice given normal drinking water (Con). (*)= $p < 0.05$ compared to PPARγ$^{F/F}$ given water containing 2.5% DSS.