

RESEARCH ARTICLES

T cell PPAR γ is required for the anti-inflammatory efficacy of abscisic acid against experimental IBD

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Received 15 March 2010; received in revised form 14 June 2010; accepted 30 June 2010

Abstract

The phytohormone abscisic acid (ABA) has been shown to be effective in ameliorating chronic and acute inflammation. The objective of this study was to investigate whether ABA's anti-inflammatory efficacy in the gut is dependent on peroxisome proliferator-activated receptor γ (PPAR γ) in T cells. PPAR γ -expressing and T cell-specific PPAR γ null mice were fed diets with or without ABA (100 mg/kg) for 35 days prior to challenge with 2.5% dextran sodium sulfate. The severity of clinical disease was assessed daily, and mice were euthanized on Day 7 of the dextran sodium sulfate challenge. Colonic inflammation was assessed through macroscopic and histopathological examination of inflammatory lesions and real-time quantitative RT-PCR-based quantification of inflammatory genes. Flow cytometry was used to phenotypically characterize leukocyte populations in the blood and mesenteric lymph nodes. Colonic sections were stained immunohistochemically to determine the effect of ABA on colonic regulatory T (T_{reg}) cells. ABA's beneficial effects on disease activity were completely abrogated in T cell-specific PPAR γ null mice. Additionally, ABA improved colon histopathology, reduced blood F4/80⁺CD11b⁺ monocytes, increased the percentage of CD4⁺ T cells expressing the inhibitory molecule cytotoxic T lymphocyte antigen 4 in blood and enhanced the number of T_{reg} cells in the mesenteric lymph nodes and colons of PPAR γ -expressing but not T cell-specific PPAR γ null mice. We conclude that dietary ABA ameliorates experimental inflammatory bowel disease by enhancing T_{reg} cell accumulation in the colonic lamina propria through a PPAR γ -dependent mechanism.

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Keywords: Abscisic acid; PPAR γ ; Regulatory T cells; Inflammatory bowel disease

1. Introduction

Inflammatory bowel disease (IBD), with its two clinical manifestations – Crohn's disease and ulcerative colitis, is characterized by significant inflammation and immune cell infiltration into the gastrointestinal tract [1]. There is a need for novel safer and more effective therapies, as the current IBD medications, including aminosalicylates, corticosteroids, immunosuppressors and anti-tumor necrosis factor α therapy, are modestly effective and often have significant side effects [2]. Activation of PPAR γ by the thiazolidinedione (TZD) rosiglitazone has shown efficacy in the treatment of human patients with ulcerative colitis [3,4]. However, TZDs are unlikely to be adopted for the treatment of IBD due to their significant side effects, including fluid retention, hepatotoxicity, weight gain and congestive heart failure [5].

Recent work in our laboratory has focused on the phytohormone abscisic acid (ABA) and its effects on inflammation in animal models of inflammation. For instance, we have demonstrated that ABA intake improves glucose tolerance and reduces white adipose tissue inflammation without discernable side effects in mouse models of obesity and diabetes [6,7]. The nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ), which is highly expressed in adipose tissue, colonic epithelial cells and immune cells, was key in this response, as the deficiency of PPAR γ in immune cells resulted in a significantly diminished response to ABA's anti-diabetic effects [7]. PPAR γ has been shown to be influential in diminishing the inflammatory response and promoting an alternative M2 macrophage phenotype [8]. In lymphocytes, it has been shown to be important in the function of CD4⁺CD25⁺FoxP3⁺ regulatory T (T_{reg}) cells [9,10] and in the regulation of colonic expression of adhesion molecules as well as recruitment of T_{reg} cells into the inductive mucosal sites [11].

We recently demonstrated that dietary ABA significantly decreases gut inflammation and colonic expression of cellular adhesion cell molecules. We also demonstrated that ABA treatment increases the expression of the immunosuppressive protein cytotoxic T lymphocyte antigen 4 (CTLA4) on the surface of PPAR γ -expressing CD4⁺ T cells. Based on these findings, we sought to determine whether T cell PPAR γ expression is required for ABA's anti-inflammatory protective actions against experimental IBD [12]. This study demonstrates for the first time that ABA-induced inhibition of dextran sodium sulfate (DSS) colitis is abolished in mice lacking PPAR γ in T cells. ABA increases blood CTLA4⁺CD4⁺ T cells and T_{reg} cell numbers in mesenteric lymph nodes (MLNs) and colons of mice expressing PPAR γ , whereas in mice that lack T cell PPAR γ , the ABA-mediated modulation of regulatory CD4⁺ T cell subsets is impaired

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Table 1
Oligonucleotide sequences for quantitative real-time PCR

Primer	Sequence	Length	Accession number
β-actinF	5'CCAGGCATTGCTGACAGG3'	141	X03672
β-actinR	5'TGGAAGGTGACAGTGAGGC3'		
PPARγF	5'CAGGCTTGCTGAACGTGAAG3'	117	NM_011146
PPARγR	5'GGAGCACCTTGGCGAACA3'		
E-selectinF	5'CAGCTTTGCATGATGGCGTCT3'	83	NM_011345
E-selectinR	5'GAAGGGTACAGCGAGTTGG3'		
VCAM-1F	5'TCTCCAGGAATACAACGAT3'	75	NM_011693
VCAM-1R	5'ACAGTCATTGTCACAGCAC3'		
MAdCAM-1F	5'CCCATGGCCACAGCTACCTCA3'	85	NM_013591
MAdCAM-1R	5'CCCTGGCCCTAGTACCCTAC3'		
MMP-9F	5'GGTGGCAGCCGACGAGTT3'	128	NM_013599
MMP-9R	5'GGATGCCGCTATGTCGTTCT3'		
IL-6F	5'TTTCCTCTGGTCTTCTGGAG3'	92	NM_031168
IL-6R	5'CTGAAGGACTCTGGCTTTGT3'		
IFNγF	5'GGGGGTGGGGGACAGC3'	140	K00083
IFNγR	5'TGGCCCGAGTGTAGACATC3'		

F indicates forward; R, reverse. PCR primer pairs were designed for an optimal annealing temperature of 57°C and product lengths between 78 and 157 base pairs. When plotting threshold cycle versus log starting quantity (pg), standard curves had slopes between -3.1 and -3.7, PCR efficiencies between 92 and 105 and R² above 0.98.

and ABA modestly exacerbates colitis. These findings demonstrate that T cell PPARγ is critical for ABA's anti-inflammatory effects in experimental IBD.

2. Materials and methods

2.1. Animal procedures

Six- to eight-week-old PPARγ fl/fl; CD4-Cre⁻ (wild-type, WT) and PPARγ fl/fl; CD4-Cre⁺ (CD4cre) T cell-specific PPARγ null mice in a C57BL/6j background (N=35) were housed at the animal facilities of the Virginia Polytechnic Institute and State University in a room maintained at 75°F, with a 12:12 h light–dark cycle starting from 6:00 a.m. All experimental procedures were approved by the Institutional Animal Care

and Use Committee of the Virginia Polytechnic Institute and State University and met or exceeded requirements of the Public Health Service/National Institutes of Health and the Animal Welfare Act. Mice undergoing *in vivo* experimental treatments were fed AIN-93G-based purified diets with or without 100 mg/kg of a racemic ABA mixture for 35 days prior to induction of colitis with water containing 2.5% DSS, 36,000–44,000 mol wt (ICN Biomedicals, Aurora, OH). Following the DSS challenge, mice were weighed on a daily basis and examined for clinical signs of disease associated with colitis (i.e., perianal soiling, rectal bleeding, diarrhea and piloerection). For the DSS challenge, the disease activity indices and rectal bleeding scores were calculated using a modification of a previously published compounded clinical score [13]. Mice in the DSS study were euthanized on Day 7 of the DSS challenge by carbon dioxide narcosis followed by secondary thoracotomy, and their blood was withdrawn from the heart. Spleen and MLNs were scored based on size (splenomegaly or adenopathy), macroscopic inflammatory lesions (0–3) were excised and single-cell suspensions were generated as previously described [9].

2.2. Histopathology

Colonic sections were fixed in 10% buffered neutral formalin, later embedded in paraffin and then sectioned (5 μm) and stained with H&E stain for histological examination. Colons were graded with a compounded histological score including the extent of (1) leukocyte infiltration, (2) mucosal thickening and (3) epithelial cell erosion. The sections were graded with a score of 0–4 for each of the previous categories, and data were analyzed as a normalized compounded score.

2.3. Immunophenotyping of blood and MLNs

MLNs were excised, crushed with frosted slides and resuspended in PBS and enumerated with a Coulter Counter (Beckman Coulter, Fullerton, CA). MLN-derived cells (2×10⁵ cells/well) or whole blood (10 μl/well) was seeded onto 96-well plates, centrifuged at 4°C at 3000 rpm for 4 min and washed with PBS containing 5% serum and 0.09% sodium azide (FACS buffer). To assess differential monocyte/macrophage subsets, we then incubated the cells in the dark at 4°C for 20 min in FcBlock (20 μg/ml; BD Pharmingen) and then for an additional 20 min with fluorochrome-conjugated primary antibodies anti-F4/80, anti-Ly6c and anti-CD11b as previously shown [14]. For lymphocyte assessment, cells were incubated with anti-CD4, anti-CD8, CD3, anti-FoxP3-PE and anti-CD152 (CTLA4) as previously shown [9,14]. Flow results were computed with a BD LSR II flow cytometer, and data analyses were performed with FACS Diva software (BD).

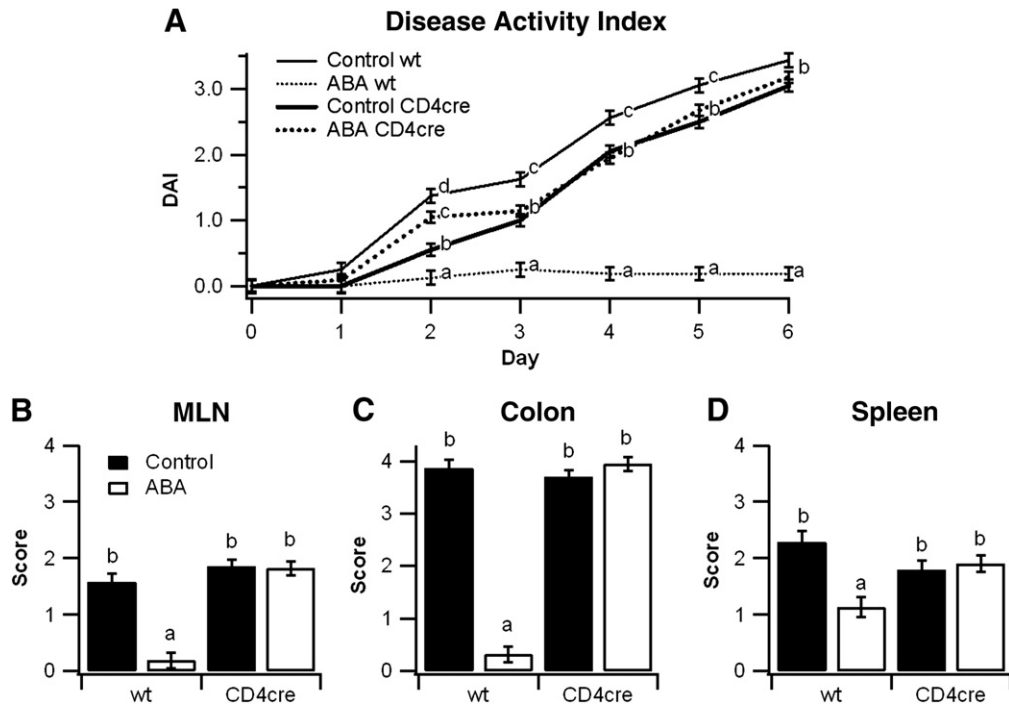


Fig. 1. Effect of dietary ABA supplementation on disease severity in PPARγ-expressing and T cell-specific PPARγ null mice. PPARγ WT and PPARγ CD4cre mice were fed ABA-supplemented (100 mg/kg) or control diets for 35 days and challenged with 2.5% DSS for 7 days. The disease activity index (DAI), a composite score reflecting clinical signs of the disease (i.e., perianal soiling, rectal bleeding, diarrhea and piloerection), was assessed daily (A). On Day 7, mice were euthanized and the colon, spleen and MLNs (B–D) were macroscopically scored for inflammation. Statistically significant differences (P<.05) between groups are indicated with different letter subscripts (n=8 mice/group) in three independent experiments.

2.4. Immunohistochemistry

Immunohistochemistry was performed as previously described [15]. T_{reg} cells were detected with anti-CD4 conjugated with FITC (1:100; eBioscience) and co-localized with anti-FoxP3 conjugated with biotin (1:100; eBioscience). Immunodetection of FoxP3 was performed using streptavidin conjugated with Alexa Fluor 546 (1 mg/L; Invitrogen, Carlsbad, CA). All images were captured with a Nikon Eclipse TE2000-U and analyzed with NIS-Elements (Nikon Instruments, Melville, NY).

2.5. Quantitative real-time RT-PCR

Total RNA was isolated from colons using a Qiagen RNA Isolation Mini Kit according to the manufacturer's instructions. Total RNA (1 µg) was used to generate complementary DNA (cDNA) template using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The total reaction volume was 20 µl, with the reaction incubated as follows in an MJ MiniCycler: 5 min at 25°C, 30 min at 52°C, 5 min at 85°C and hold at 4°C. PCR was performed on the cDNA using Taq DNA polymerase (Invitrogen) and using previously described conditions [13]. Each gene amplicon was purified with the use of a MiniElute PCR Purification Kit (Qiagen) and quantitated on an agarose gel by using a DNA mass ladder (Promega). These purified amplicons were used to optimize real-time PCR conditions and to generate standard curves in the real-time PCR assay. Primer concentrations and annealing temperatures were optimized for the iCycler iQ system (Bio-Rad) for each set of primers using the system's gradient protocol.

PCR efficiencies were maintained between 92% and 105% and correlation coefficients above 0.98 for each primer set during optimization and during the real-time PCR of sample DNA.

cDNA concentrations for genes of interest were examined by real-time quantitative PCR using an iCycler iQ system and the iQ SYBR Green Supermix (Bio-Rad). A standard curve was generated for each gene using 10-fold dilutions of purified amplicons starting at 5 pg of cDNA and used later to calculate the starting amount of target cDNA in the unknown samples. SYBR green I is a general double-stranded DNA intercalating dye and may therefore detect nonspecific products and primer/dimers in addition to the amplicon of interest. In order to determine the number of products synthesized during the real-time PCR, we performed a melting curve analysis on each product. Real-time PCR was used to measure the starting amount of nucleic acid of each unknown sample of cDNA on the same 96-well plate. Results were normalized to the housekeeping gene β -actin for each sample; accession numbers for each gene examined are outlined in Table 1.

2.6. Statistics

Data were analyzed as a 2×2 factorial arrangement within a completely randomized design. To determine the statistical significance of the model, we performed analysis of variance using the general linear model procedure of Statistical Analysis Software (SAS), and $P < .05$ was considered to be significant. When the model

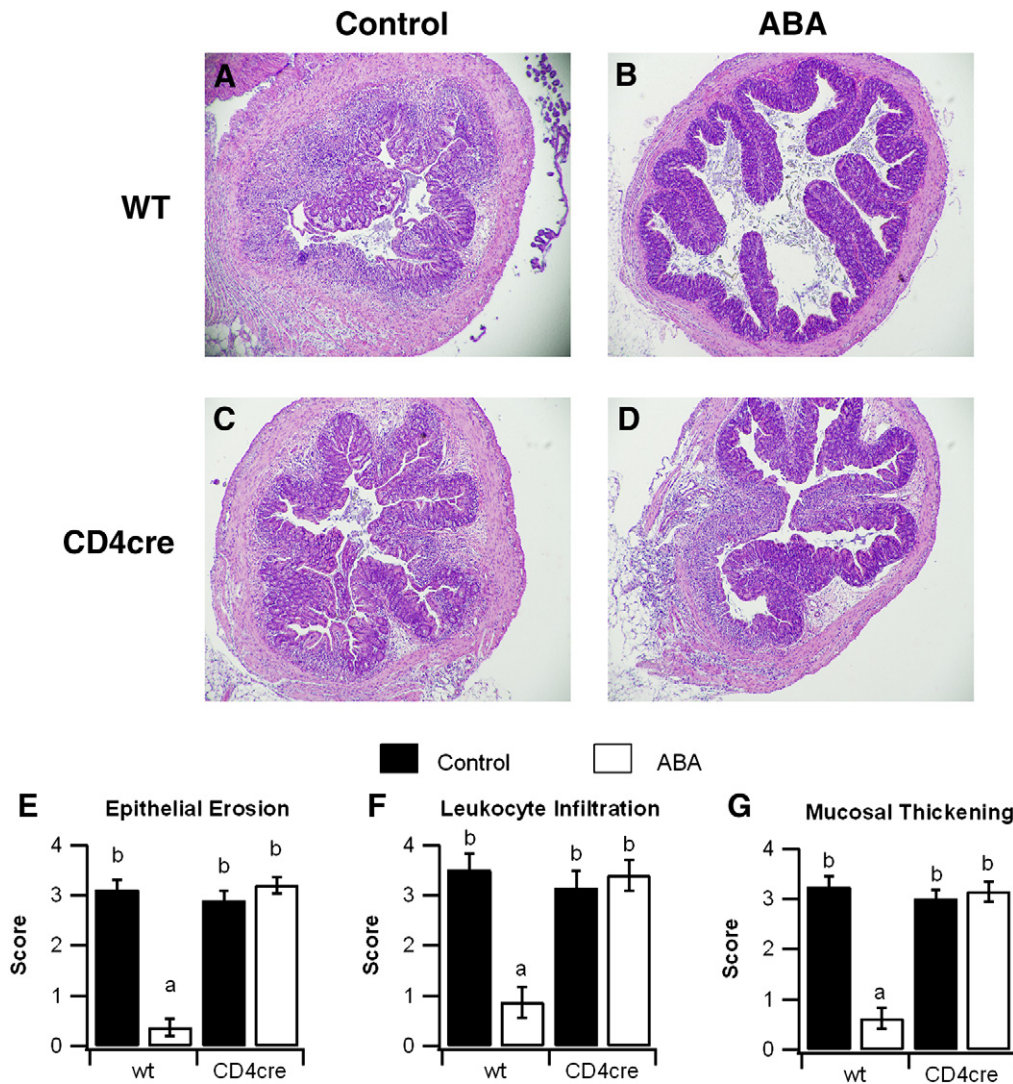


Fig. 2. Effect of dietary ABA supplementation on colon histopathology in PPAR γ -expressing and T cell-specific PPAR γ null mice. PPAR γ WT and PPAR γ CD4cre mice were fed ABA-supplemented (100 mg/kg) or control diets for 35 days and challenged with 2.5% DSS (DSS) or water (no DSS) for 7 days. Representative photomicrographs from the WT, control (A), WT, ABA (B), CD4cre, control (C) and CD4cre, ABA (D) groups are illustrated (original magnification, $\times 40$). All specimens underwent blinded histological examination and were scored (1–4) on epithelial erosion (E), mucosal wall thickening (F) and leukocyte infiltration (G). Data are represented as mean \pm standard error. Statistically significant differences ($P < .05$) between groups are indicated with different letter subscripts ($n = 8$ mice/group) in three independent experiments.

was significant, analysis of variance was followed by Fisher's Protected Least Significant Difference multiple comparison method.

3. Results

3.1. The deficiency of PPAR γ in T cells abrogates the beneficial effects of ABA on the severity of experimental IBD

We have previously demonstrated that dietary supplementation with ABA significantly reduces obesity-related inflammation in adipose tissue, T cell and macrophage infiltration in aortic atherosclerotic plaques and colonic inflammation [6,12,16]. Our objective in this study was to investigate whether T cell PPAR γ is an essential mediator of ABA's anti-inflammatory mechanism. PPAR γ -expressing and T cell-specific PPAR γ -deficient mice (CD4cre) mice were fed diets with or without ABA (100 mg/kg) for 35 days prior to a 7-day challenge with DSS. Throughout the challenge period, disease severity

was significantly reduced by dietary ABA in PPAR γ -expressing mice but not in T cell-specific PPAR γ null mice (Fig. 1A). On Day 7, post-DSS challenge colons, spleens and MLNs also showed significantly fewer macroscopic gross lesions (i.e., splenomegaly and adenopathy) in PPAR γ -expressing mice fed with ABA-supplemented diets in comparison with the other treatment groups (Fig. 1B–D).

3.2. ABA improves colonic histopathology in PPAR γ -expressing but not in T cell-specific PPAR γ null mice

We next examined the effect of ABA and T cell-specific PPAR γ deficiency on histopathological colonic inflammatory lesions. In line with our observations of disease severity and gross lesions, ABA's anti-inflammatory effects were significantly attenuated in mice lacking PPAR γ in T cells (Fig. 2). Histopathological scores of epithelial erosion, leukocyte infiltration and mucosal thickening were

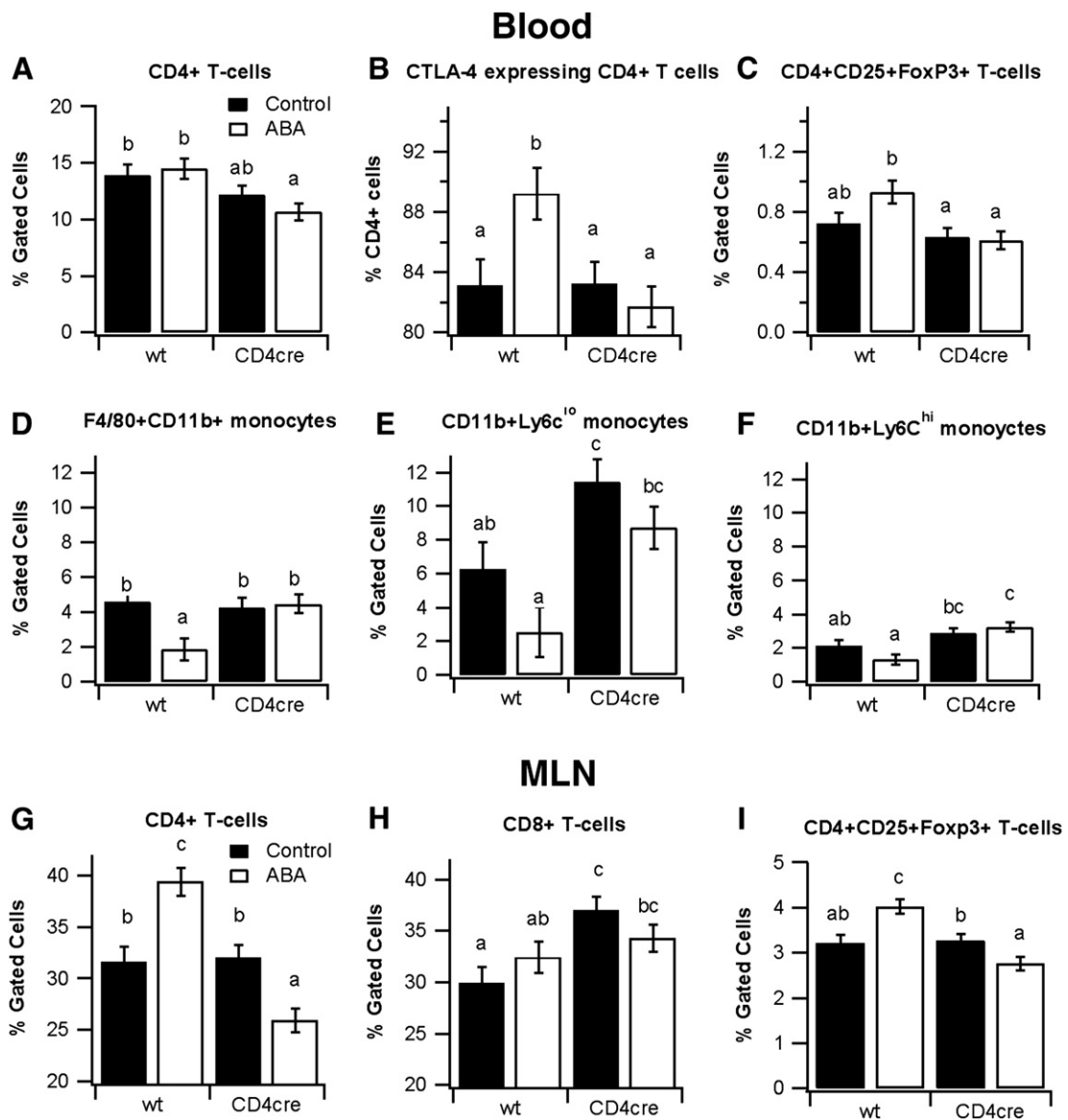


Fig. 3. Effect of dietary ABA supplementation on lymphocyte subsets in PPAR γ -expressing and T cell-specific PPAR γ null mice. PPAR γ WT and PPAR γ CD4cre mice were fed ABA-supplemented (100 mg/kg) or control diets for 35 days and challenged with 2.5% DSS for 7 days. Blood (A–C) and MLNs (D and E) were immunophenotyped with anti-CD3, anti-CD4, anti-CD8, anti-CTLA4, anti-FoxP3, anti-F4/80 and anti-CD11b mouse monoclonal antibodies, and the immune cell populations were analyzed by flow cytometry with FACS diva software. Data are represented as mean \pm standard error. Statistically significant differences ($P < .05$) between groups are indicated with an asterisk ($n = 8$ mice/group) in three independent experiments.

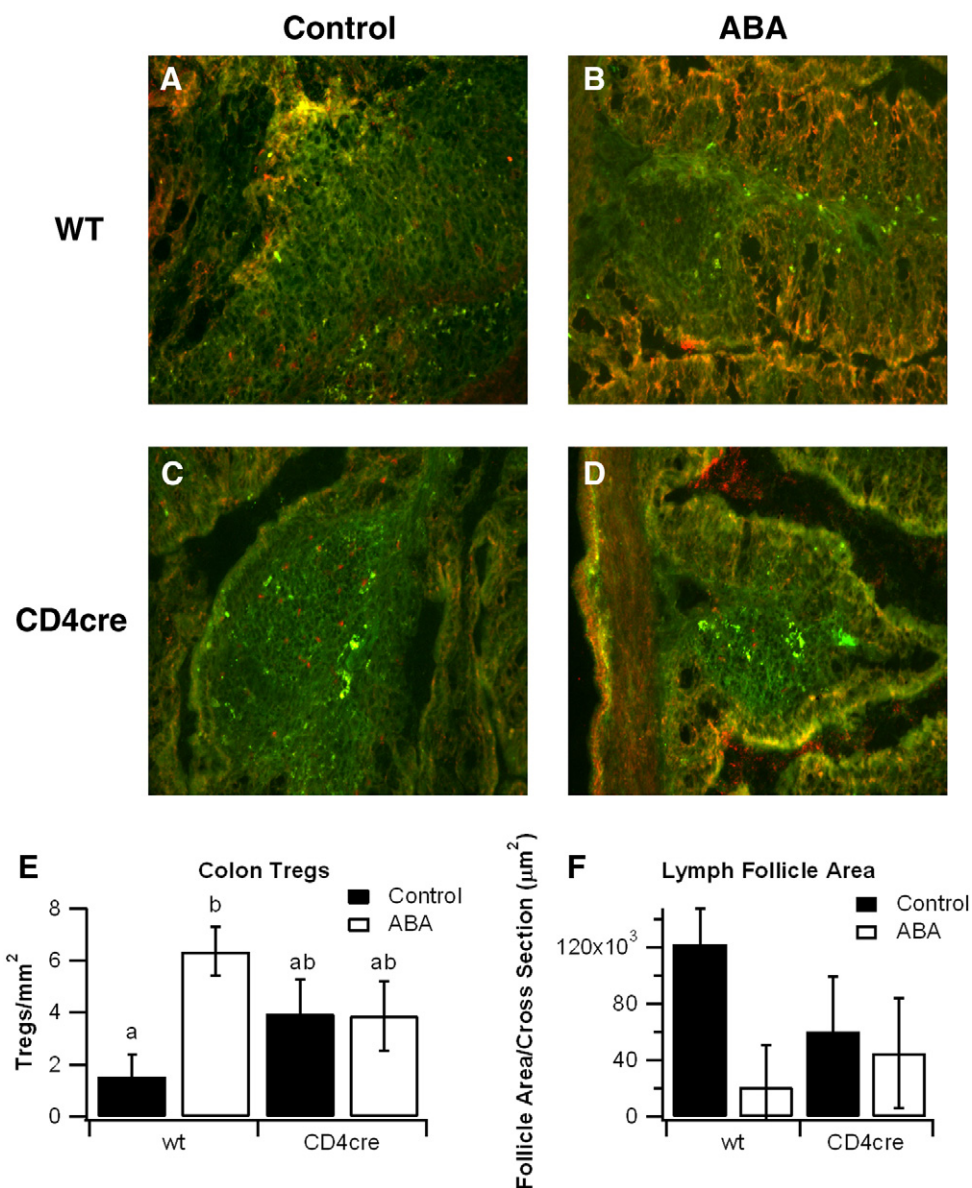


Fig. 4. Effect of dietary ABA supplementation on colonic T_{reg} cells in PPAR γ -expressing and T cell-specific PPAR γ null mice. PPAR γ WT and PPAR γ CD4cre mice were fed ABA-supplemented (100 mg/kg) or control diets for 35 days and challenged with 2.5% DSS for 7 days. T_{reg} cells were detected with anti-CD4 conjugated with FITC (1:100) and co-localized with anti-FoxP3 conjugated with biotin (1:100). Immunodetection of FoxP3 was performed using streptavidin conjugated with Alexa Fluor 546 (1 mg/L). Representative images from the WT, control (A), WT, ABA (B), CD4cre, control (C) and CD4cre, ABA (D) groups are depicted (original magnification, $\times 200$). The number of colonic T_{reg} cells (E) and the lymphoid follicle areas (F) were assessed for each group. Data are represented as mean \pm standard error. Statistically significant differences ($P < .05$) between groups are indicated with different letter subscripts ($n = 8$ mice/group) in three independent experiments.

significantly reduced in ABA-fed PPAR γ -expressing mice, although this effect was abrogated in mice lacking PPAR γ in T cells (Fig. 2).

3.3. Dietary ABA reduces blood monocytes and enhances the numbers of CTLA4-expressing and regulatory CD4⁺ T cells in blood and MLNs of PPAR γ -expressing mice

To more closely investigate the effect of ABA on immune cells, we phenotypically characterized monocyte/macrophage and lymphocyte subsets in blood and MLNs. In blood, ABA modestly increased CD4⁺CD25⁺FoxP3⁺ T_{reg} cells and significantly enhanced the percentage of CD4⁺ T cells expressing the inhibitory surface CTLA4 (CD152) in PPAR γ -expressing mice. The percentage of CD4⁺ T cells and that of T_{reg} cells were both significantly reduced in the T cell-specific PPAR γ null mice in comparison with PPAR γ -expressing mice.

Dietary ABA supplementation also significantly reduced the percentage of F4/80⁺CD11b⁺ blood monocytes in PPAR γ -expressing mice; however, this effect was impaired in the T cell-specific PPAR γ -deficient mice. We also examined the percentage of CD11b⁺Ly6c^{lo} (Ly6c^{lo}) and that of CD11b⁺Ly6c^{hi} (Ly6c^{hi}) monocytes. Ly6c^{hi} monocytes have been noted for high expression of CCR2 and a propensity for differentiating into M1 pro-inflammatory macrophages, whereas Ly6c^{lo} monocytes, which highly express the fractalkine receptor CX3CR1, represent a more mature phenotype with greater phagocytic capacity [17]. Here, both Ly6c^{hi} and Ly6c^{lo} monocytes were significantly up-regulated in T cell-specific PPAR γ null mice compared with PPAR γ -expressing mice. In addition, dietary ABA caused a decrease in numbers of both monocyte subsets. In MLNs, dietary ABA supplementation significantly enhanced the percentage of CD4⁺ T cells and that of T_{reg} cells in PPAR γ -expressing mice, and it exerted the reverse effect in tissue-specific PPAR γ null mice (Fig. 3G–I).

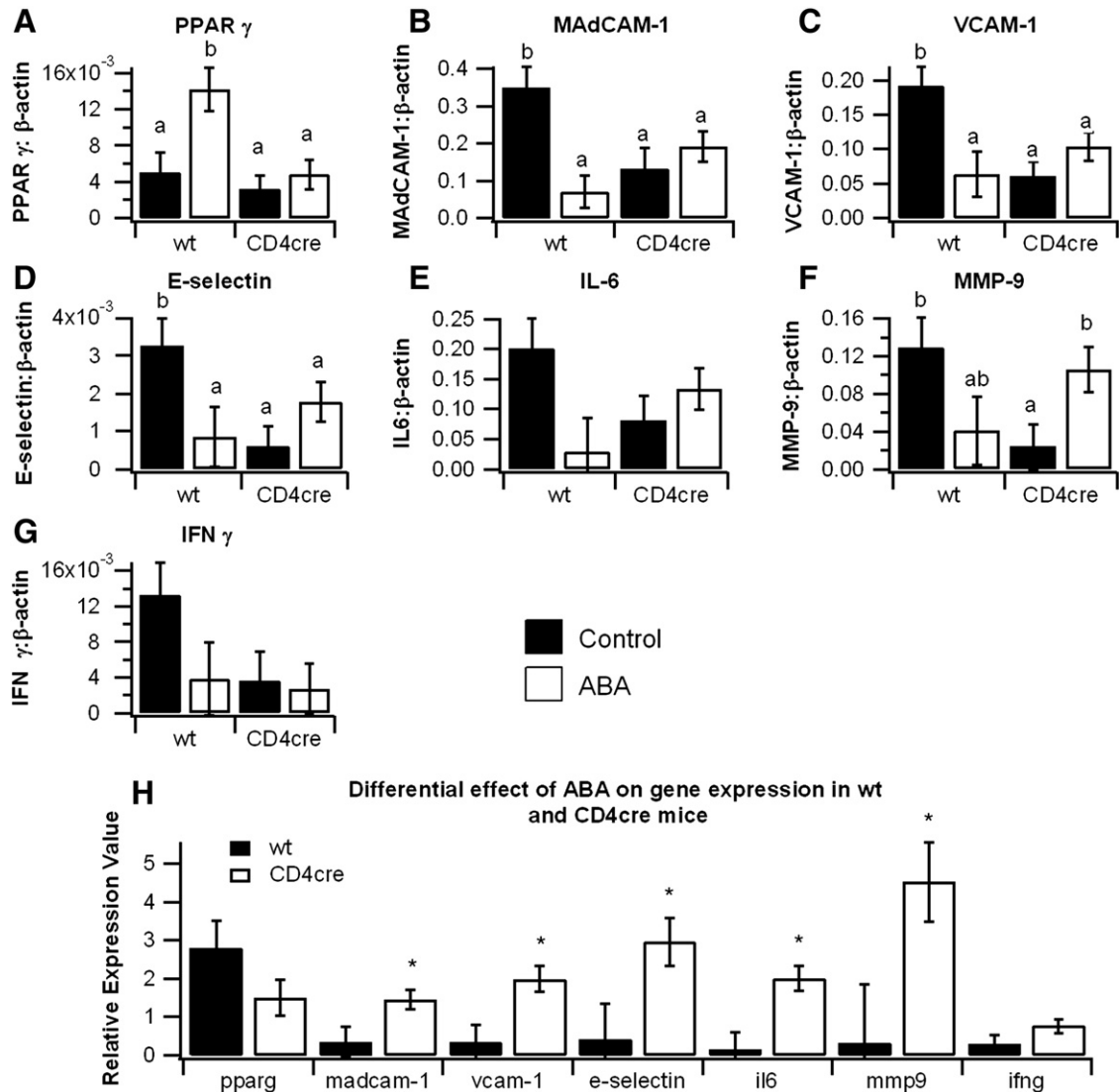


Fig. 5. Differential effect of ABA on colon gene expression in PPAR γ -expressing and T cell-specific PPAR γ -deficient mice. PPAR γ WT and PPAR γ CD4cre mice were fed ABA-supplemented (100 mg/kg) or control diets for 35 days and challenged with 2.5% DSS for 7 days. Expressions of (A) PPAR γ , (B) MAdCAM-1, (C) VCAM-1, (D) E-selectin, (E) IL-6, (F) MMP-9 and (G) interferon γ (IFN γ) were quantified by real-time quantitative RT-PCR. Statistically significant differences ($P < .05$) between groups are indicated with different letter subscripts ($n = 8$ mice/group) in three independent experiments. (H) The effect of ABA treatment on colonic gene expression for each genotype was calculated as a relative expression value with respect to the control treatment. Data are represented as mean \pm standard error. Points with an asterisk are significantly different ($P < .05$). Statistically significant differences ($P < .05$) between groups are indicated with an asterisk ($n = 8$ mice/group) in three independent experiments.

3.4. Dietary ABA increases T_{reg} cell numbers in the colonic mucosa

Our data indicate that ABA's anti-inflammatory effects are significantly mitigated in mice deficient in T cell PPAR γ . PPAR γ has been shown to be important in the function of T_{reg} cells [9,18], which serve as down-regulators of inflammation in the gastrointestinal tract [19,20]. We found here that ABA significantly increases the number of T_{reg} cells in the colonic lamina propria of PPAR γ -expressing mice, an effect that is attenuated in the colons of T cell-specific PPAR γ null mice (Fig. 4). ABA also caused a numeric reduction in the average size of lymphoid follicles in PPAR γ -expressing mice (Fig. 4).

3.5. Dietary ABA down-regulates expression of adhesion molecules and pro-inflammatory cytokines in PPAR γ -expressing but not in T cell-specific PPAR γ -deficient mice

We have previously shown that dietary ABA supplementation reduces expression of adhesion molecules vascular adhesion mole-

cule 1 (VCAM-1), mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) and E-selectin and pro-inflammatory cytokines interleukin 6 (IL-6) and matrix metalloproteinase-9 (MMP-9) in colons of DSS-treated mice [11,12]. Here, we used real-time RT-PCR to investigate the differential effect of ABA on gene expressions in colons of PPAR γ -expressing and T cell-specific PPAR γ null mice. ABA inhibited mRNA expression of adhesion molecules and pro-inflammatory cytokines in WT mice. In contrast, dietary ABA supplementation resulted in a net increase of inflammatory markers in colons of T cell-specific PPAR γ null mice (Fig. 5). Specifically, mRNA concentrations of MAdCAM-1, VCAM-1, E-selectin, IL-6 and MMP-9 were all elevated in ABA-fed T cell-specific PPAR γ null mice in comparison with control-fed mice (Fig. 5).

4. Discussion

ABA is a phytohormone involved in plant growth, response to stress dormancy and life cycle that was discovered and chemically

synthesized in 1965 [21]. Interestingly, recent evidence demonstrates that ABA exerts pleiotropic effects in a number of animal models of inflammatory diseases [22]. For instance, dietary ABA has shown efficacy in suppressing inflammatory macrophage accumulation into abdominal white adipose tissue, thereby ameliorating peripheral insulin resistance and inflammation in mouse models of type II diabetes and obesity [6,7,23]. ABA at nanomolar concentrations acts locally on human pancreatic β -cells and enhances their insulin-secreting ability [24]. In addition, dietary ABA ameliorates atherosclerotic plaque formation in the aortas of ApoE^{-/-} mice by suppressing macrophage and CD4⁺ T cell infiltration into the intima and media layers of the aortic root walls [16]. In line with these findings indicating anti-inflammatory efficacy of ABA disease models, more recently, we have demonstrated that dietary ABA prevents or ameliorates colonic inflammation in a mouse model of experimental IBD [12]. This study demonstrated that ABA treatment significantly reduces adhesion molecule expression and colitis, and *in vitro* examination of cultured splenocytes indicated that PPAR γ -dependent up-regulation of CTLA4 expression on T cells may be involved in ABA's immunomodulatory mechanism [12]. Thus, the objective of this study was to examine whether the deficiency of T cell PPAR γ significantly impairs ABA's effect on DSS colitis severity.

PPAR γ -expressing or T cell-specific PPAR γ -deficient mice were fed diets with or without ABA (100 mg/kg) for 35 days prior to a 7-day induction of colitis. The results from the DSS challenge demonstrated that the absence of PPAR γ in T cells essentially abolishes the ability of ABA to decrease experimental IBD, including the anti-inflammatory action of ABA in the colon. Of note, ABA increased the numbers of T_{reg} cells in MLNs and the colonic lamina propria. Interestingly, this effect of ABA was abrogated in T cell-specific PPAR γ null mice, indicating that T cell PPAR γ was required for the regulation of T_{reg} cell number in the mucosal inductive (MLNs) and effector (colonic lamina propria) sites. The finding that ABA's anti-inflammatory effects are dependent on functional PPAR γ in immune cells is in line with our previous findings in a model of diet-induced obesity in which the absence of PPAR γ in hematopoietic and epithelial cells significantly impaired ABA-induced improvements in glucose tolerance and adipose tissue macrophage infiltration [7].

One factor possibly contributing to the improvements by ABA was the enhancement of CTLA4 expression on CD4⁺ T cells. We had previously reported that ABA increases CTLA4 expression *in vitro* in CD3/CD28-stimulated CD4⁺ T cells in a PPAR γ -dependent manner [12]. CTLA4 is a molecule involved in down-regulation of T cell responses that is highly expressed by naturally occurring CD4⁺CD25⁺ T_{reg} cells [25]. The importance of PPAR γ in T_{reg} cell function is well established [9,10], as too is the importance of CTLA4 for the *in vivo* functional effects of T_{reg} cells [26,27]. Thus, ABA may enhance T_{reg} cell numbers and their function by activating PPAR γ . Further studies are warranted to dissect ABA's effect on effector versus T_{reg} cells during chronic intestinal inflammation in adoptive co-transfer models of T cell-induced colitis.

Dietary ABA worsened colonic inflammation and enhanced cellular adhesion molecule expression in T cell-specific PPAR γ null mice. The finding that ABA can worsen inflammation in mammalian cells is not surprising since studies by Bruzzone *et al.* [28] and Magnone *et al.* [29] have shown that ABA exerts pro-inflammatory effects in human granulocytes and monocytes *in vitro*, and these authors have suggested that ABA may act as an endogenously generated pro-inflammatory signaling molecule. Specifically, Magnone *et al.* [29] posited that ABA evokes an intracellular calcium rise through the second messenger cyclic ADP-ribose, leading to nuclear factor- κ B activation in monocytes. Our findings in several studies [6,7,12,16,23] do not support their conclusion but suggest rather that T cell PPAR γ is important in ABA's systemic and mucosal anti-inflammatory effects *in vivo*. Nonetheless, since ABA treatment worsened the colitis severity in

mice lacking PPAR γ in T cells, it is tempting to speculate that ABA-mediated activation of anti-inflammatory pathways predominates when PPAR γ is expressed in T cells, whereas pro-inflammatory pathways, such as nuclear factor- κ B, become ABA's targets when PPAR γ is deleted from T cells. Interestingly, T cell-specific PPAR γ null mice did not respond to oral ABA administration despite expressing PPAR γ in macrophages, dendritic cells, neutrophils, endothelial cells and epithelial cells – all of which contribute to the pathogenesis of DSS colitis. Together, these findings indicate that T cell PPAR γ is a crucial mediator of ABA's anti-inflammatory responses. More investigation is needed to characterize the cell specificity of ABA's immunomodulatory mechanisms.

Another aspect that remains unknown is how ABA interacts with PPAR γ to suppress inflammation. There are currently data showing that ABA may act through the lanthionine synthetase C-like 2 protein to increase intracellular levels of cAMP, CD38-produced cADP-ribose and calcium [30]. Recent findings from our laboratory suggest that PPAR γ activation by ABA may occur downstream of the cAMP/PKA signaling axis [23]. We have also identified the binding site of PPAR γ agonists, including ABA, on the lanthionine synthetase C-like 2 protein [31]. Further insight into this response may be key to understanding the cell-specific response of ABA-induced PPAR γ activation and how it resembles and differs from the mechanism of PPAR γ activation of the TZD class of anti-diabetic drugs that is associated with significant adverse side effects as described in Section 1.

In conclusion, we found that dietary ABA supplementation significantly ameliorates disease activity and colonic inflammatory lesions during experimental IBD through a T cell PPAR γ -dependent mechanism. This study suggests that ABA may be effective as a therapeutic or prophylactic agent for IBD and other autoimmune disorders in which T cell-mediated immunoregulation exerts a beneficial effect.

Acknowledgments

This study was supported by the National Center for Complementary and Alternative Medicine at the National Institutes of Health (Award Number 5R01AT4308 awarded to J.B.-R.), the European Commission (Grant Number 224836), the Ramon y Cajal Program and funds from the Nutritional Immunology and Molecular Nutrition Laboratory.

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