The American Journal of Pathology, Vol. 169, No. 6, December 2006

DOI: 10.2353/ajpath.2006.060186

Gastrointestinal, Hepatobiliary and Pancreatic Pathology

Signal Transducer and Activator of Transcription 5b Promotes Mucosal Tolerance in Pediatric Crohn's Disease and Murine Colitis

Xiaonan Han,* Bankole Osuntokun,* Nancy Benight,* Kimberly Loesch,† Stuart J. Frank,†‡§ and Lee A. Denson*

From the Department of Pediatrics, Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, Ohio; the Departments of Cell Biology‡ and Medicine,‡ University of Alabama, Birmingham, Alabama; and the Endocrinology Section,§ Medical Service, Birmingham Veterans Affairs Medical Center, Birmingham, Alabama*

Growth hormone (GH) regulates anabolic metabolism via activation of the STAT5b transcription factor and reduces mucosal inflammation in colitis. Peroxisome proliferator-activated receptor (PPAR) γ suppresses mucosal inflammation and is regulated by GH through STAT5b. We hypothesized that the GH:STAT5b axis influences susceptibility to colitis via regulation of local PPARγ abundance. Colon biopsies from children with newly diagnosed Crohn's disease (CD) and controls were exposed to GH in short-term organ culture. Trinitrobenzene sulfonic acid (TNBS) administration was used to induce colitis in STAT5b-deficient mice and wild-type controls, with and without rosiglitazone pretreatment. GH receptor, STAT5b, PPARγ, and nuclear factor κB activation and expression were determined. Epithelial cell GH receptor expression and GH-dependent STAT5b activation and PPARγ expression were reduced in CD colon. STAT5b-deficient mice exhibited reduced basal PPARγ nuclear abundance and developed more severe proximal colitis after TNBS administration. This was associated with a significant increase in mucosal nuclear factor κB activation at baseline and after TNBS administration. Rosiglitazone ameliorated colitis in wild-type mice but not STAT5b-deficient mice. GH-dependent STAT5b activation is impaired in affected CD colon and contributes to chronic mucosal inflammation via down-regulation of local PPARγ expression. Therapeutic activation of the GH:STAT5b axis therefore represents a novel target for restoring both normal anabolic metabolism and mucosal tolerance in CD. ***(Am J Pathol 2006, 169:1999–2013; DOI: 10.2353/ajpath.2006.060186)***

Current evidence suggests that Crohn's disease (CD) is caused by loss of tolerance to the enteric flora, leading to chronic inflammation and intestinal damage.[1] Increased epithelial nuclear factor κB (NF-κB) activity and consequent chemokine expression are fundamental molecular features of this loss of tolerance. Increased NF-κB DNA-binding activity has been attributed to increased binding of the p50 and p65 subunits.[2] Importantly, disease activity in mice with colitis due to 2,4,6-trinitrobenzene sulfonic acid (TNBS) administration was inhibited by antisense oligonucleotides that inhibit p65 expression.[2] These studies suggest a critical role for NF-κB in mediating expression of proinflammatory factors, which are central to the pathogenesis of CD. Although much less is known regarding the regulatory transcription factors that may promote mucosal tolerance, recent studies have suggested that both STAT5a/b and peroxisome proliferator-activated receptor (PPAR) γ may play a role.

Colonic mRNA expression of the STAT5b transcription factor is down-regulated in affected segments in adults with CD and ulcerative colitis.[3] However, the functional significance of this is not known. Moreover, whether GH-dependent STAT5b tyrosine phosphorylation and DNA binding are reduced in affected CD colon has not been determined. Current data suggest that the closely related STAT5a and STAT5b transcription factors are required, in

Supported by National Institutes of Health (NIH) grants DK02700 and DK63956, the Cincinnati Children's Hospital Research Foundation, and the Crohns and Colitis Foundation of America (to X.H. and L.A.D.); the Children's Digestive Health Foundation/Nestle Nutrition and the Broad Medical Research Program (to L.A.D.); and NIH grant DK058259 (to S.J.F.). Patient-based studies were supported by United States Public Health Service grant MO1 RR 08084, General Clinical Research Centers Program, National Center for Research Resources, NIH.

Accepted for publication August 14, 2006.

Address reprint requests to Lee A. Denson, M.D., MLC 2010, 3333 Burnet Ave., Cincinnati, OH 45229-3039. E-mail: lee.denson@cchmc.org.

a largely redundant manner, for the survival of anti-inflammatory regulatory T cells (Treg) and maintenance of mucosal tolerance. Combined deletion of STAT5a and STAT5b results in reduced survival of Treg and the spontaneous development of autoimmune disease including colitis.[4] Targeted deletion of STAT5b alone results in growth failure due to impaired growth hormone (GH) signaling, without spontaneous autoimmune disease.[5–8] A nonredundant role for STAT5b in preventing mucosal inflammation has not previously been proposed. Although our group and others have shown that GH administration will improve symptoms in patients with CD and reduce mucosal inflammation in several animal models of colitis, whether this involves mucosal STAT5b activation is not known.[9–14]

PPARγ is a nuclear receptor that is highly expressed in colon epithelial cells (CECs) and lamina propria macrophages (LPMs) and that promotes mucosal tolerance by inhibiting NF-κB activation.[15,16] Recently, PPARγ has been shown to inhibit NF-κB activity by associating with the p65 subunit in the nucleus and promoting export of the PPARγ:p65 complex to the cytosol.[16] Multiple experimental studies using PPARγ haplo-insufficient mice and PPARγ gene therapy have confirmed a profound anti-inflammatory effect in murine colitis.[15,17–19] Patient-based studies have demonstrated a reduction in PPARγ expression in inflamed colon, with mixed results obtained with PPARγ ligand administration.[20–23] Administration of the PPARγ agonist rosiglitazone has also been more consistently effective when given before the onset of experimental colitis than when given after the onset of disease.[15,18,19,24] This may be due to the basal reduction in PPARγ nuclear abundance, preventing an optimal response to ligand administration. The molecular basis for the reduction in PPARγ expression in colitis has not been defined. Recently, GH has been shown to regulate the human PPARγ3 transcript, which is highly expressed in CECs and LPMs, via a STAT5b *cis*-element.[25] Interestingly, STAT5a could not substitute for STAT5b in this regard. Whether STAT5b, via regulation of PPARγ nuclear abundance, would exert a nonredundant tolerogenic effect in colitis was not known.

In this study, we have determined that STAT5b activation and PPARγ expression are reduced in pediatric CD colon at diagnosis. STAT5b-deficient mice exhibited reduced colonic PPARγ nuclear abundance, increased NF-κB activation, and enhanced susceptibility to colitis after TNBS administration; this was refractory to rosiglitazone administration.

Materials and Methods

Human GH was from Sigma (St. Louis, MO). All antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA) unless otherwise noted. Anti-GH receptor $(GHR)_{cyt\text{-}AL47}$ is a polyclonal antibody against the human GHR cytoplasmic domain that cross reacts with mouse GHR.[26] This serum and its control preimmune serum were purified by ammonium sulfate precipitation. Antibodies specific for STAT5a and STAT5b were from Zymed Laboratories (South San Francisco, CA). A tyrosine phosphorylation-specific STAT5 antibody was from Upstate Biotechnology (Lake Placid, NY). Rosiglitazone was from Cayman Chemicals (Ann Arbor, MI) and was blended with the chow food at 80 mg/kg food to provide 20 mg/kg/day to a 20-g mouse consuming 5 g of chow food per day. We confirmed that wild-type (WT) and STAT5b-deficient mice consumed sufficient chow in the 3 days before TNBS administration to receive 20 mg/kg rosiglitazone per day.

Patient-Based Studies

Colon biopsies were obtained from untreated pediatric patients with Crohn's colitis and healthy controls 5 to 18 years of age during the initial diagnostic colonoscopy. Biopsies were obtained from endoscopically affected segments primarily in the cecum and ascending colon. The diagnosis of CD was made by the patient's primary gastroenterologist based on established clinical, radiological, and histological criteria. The study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board and the Cincinnati Children's Hospital Medical Center General Clinical Research Center Scientific Advisory Committee. The Crohn's Disease Histological Index of Severity (CDHIS) was computed as previously described by a single gastrointestinal pathologist (E.B.).[27,28]

Human Colon Biopsy Organ Culture

In brief, colon biopsies were washed with cold phosphate-buffered saline (PBS) and trimmed into explants.[10] Explants were cultured in serum-free CMRL-1066 tissue culture medium (GIBCO, Grandland, NY) on collagen I-coated plates (BIOCOAT, Bedford, MA) at 37°C in 95% oxygen, 5% carbon dioxide atmosphere for 24 hours. The morphological appearance of the explants was preserved under these conditions as assessed by light microscopy. After 24 hours in culture, explants were treated with human GH (500 ng/ml) or PBS, and nuclear protein and sections were prepared 45 minutes later. This dose of human GH has previously been shown to regulate chloride secretion in the T84 human colon carcinoma cell line and to activate STAT5 in liver cell lines.[29,30]

Animal Resources and Maintenance

STAT5b-deficient mice were provided by Dr. James Ihle (St. Jude Children's Research Hospital, Memphis, TN).[8] These mice are on a C57BL6/J background; sex-matched males and females were used between 8 and 12 weeks of age. The protocol was approved by the Cincinnati Children's Hospital Research Foundation Institutional Animal Care and Use Committee.

TNBS Colitis

TNBS colitis was induced as previously described.[31,32] One or 2 mg of TNBS in 50% ethanol was administered in

a total volume of 100 μl, as indicated. Controls received the same volume of PBS or 50% ethanol. Mice also received 20 mg/kg rosiglitazone per day mixed with the chow for 3 days before and daily after induction of colitis, as indicated. Mice were sacrificed 4 days after TNBS administration. Endoscopic disease severity was assessed on day 4 using the STORZ Coloview endoscope.[33] The colon was divided into equal proximal and distal segments and stained with hematoxylin and eosin (H&E). Histological disease activity was assessed in a blinded fashion using the system reported by Neurath et al.[32]

RNA Isolation, Laser Capture Microdissection (LCM), and Real-Time Polymerase Chain Reaction (PCR)

For isolation of whole-colon RNA, human colon biopsies saved in RNAlater or longitudinal sections of mouse colon were homogenized in lysis buffer by mechanical homogenizer (Fisher Scientific Co., Pittsburgh, PA), and total RNA was isolated with RNeasy Mini kit (human colon) (Qiagen, Hilden, Germany) or Trizol (mouse colon). The quality and concentration of the total RNA were measured and checked by the Agilent Bioanalyzer 2100 (Hewlett Packard) using the RNA 6000 Nano Assay, looking for a 28S/18S ratio of 1.6 to 2.0. Colon biopsies embedded in OCT were sectioned at 5 μm with cryostat microtome and stained with H&E. Approximately 200 crypts and adjacent surface epithelial cells were captured into caps (Arcturus, Mountain View, CA) through Arcturus Pixel II LCM apparatus, and RNA was isolated with PicoPure RNA Isolation kit (Arcturus). Total RNA (200 ng) was used to reversely transcribe to cDNA with the ProSTAR reverse transcription (RT)-PCR kit (Stratagene, La Jolla, CA); cDNA was measured with Oligreen ssDNA Quantitation reagent and kit (Molecular Probes, Eugene, OR). cDNA (100 ng) was used to do SYBR Green real-time PCR on the Mx4000 multiplex quantitative PCR instrument (Stratagene) following the Brilliant SYBR Green QPCR Master Mix manual (Stratagene). The primers used for the SYBR Green RT-PCR assay were as follows: PPARγ: sense, 5′-CCTGATAGGCCCCACTGTGT-3′, and antisense, 5′-CAGGTGGGAGTGGAACAAT-3′; GHR: sense, 5′-TTTGGAATATTTGGGCTAACAGTG-3′, and antisense, 5′-TCACCTCCTCTAATTTTCCTTCCTT-3′; and glyceraldehyde-3-phosphate dehydrogenase: sense, 5′-TTTGGCTACAGCAACAGGGTG-3′, and antisense, 5′-GGGTCTCTCTCTTCCTCTTGTGC-3′.[23,34] Amplification of glyceraldehyde-3-phosphate dehydrogenase was performed to standardize the quantification of target cDNA.

Immunoblot, Immunoprecipitation, and EMSA

Cytosolic and nuclear protein was prepared using the NE-PER kit per the manufacturer's recommendations (Pierce, Rockford, IL). Nuclear proteins (100 μg) were immunoprecipitated with the anti-p65 or anti-PPARγ antibodies and 40 μl of suspended protein A/G agarose per the manufacturer's recommendations (Santa Cruz Biotechnology). Immunoblots were performed as previously described.[10] Autoradiographs were captured using a Perfection 3200 Photo scanner (Epson America Inc., Long Beach, CA) and band intensities were quantified by densitometry and expressed as mean area density using ImageQuant (Molecular Dynamics, Sunnyvale, CA). Ten micrograms of nuclear protein was used to do STAT5 and NF-κB electrophoretic mobility shift assay (EMSA). STAT5 nuclear binding was performed using a duplex oligonucleotide probe based on GH-induced STAT5b DNA-binding element in the insulin-like growth factor (IGF)-1 gene promoter. EMSA assay for NF-κB nuclear binding was performed using a duplex oligonucleotide probe based on the NF-κB binding site upstream of the murine iNOS promoter. The oligonucleotide sequences were STAT5: sense, 5′-GGGCCTTCCTGGAAGAAA-3′, and antisense, 5′-TTTCTTCCAGGAAGGCCC-3′; and NF-κB: sense, 5′-AGCTCTCTCGGAAAGTCCCCTCTG-3′, and antisense, 5′-AGCTCAGAGGGGACTTTCCGAGAG-3′.[35,36] DNA strands were respectively labeled by biotin (Pierce), and STAT5 DNA binding was detected according to LightShift chemiluminescent EMSA kit (Pierce). For supershift, 10 μg of NF-κB p65, p50, or STAT5a/b antibody (Santa Cruz Biotechnology or Zymed) was added to the gel shift reaction after preincubation of the probe and nuclear proteins on ice. Cold competition studies used a 200-fold excess of the unlabeled oligonucleotide. Band intensities were quantified as mean area density using ImageQuant (Molecular Dynamics).

Immunohistochemistry and Immunofluorescence

In brief, deparaffined or fixed frozen sections were incubated with primary antibodies as follows: mouse or rabbit anti-pSTAT5, rabbit anti-p65, anti-GHR, and anti-PPARγ.[10] Sections were also double stained with rabbit anti-pSTAT5 and mouse anti-CD68 or rabbit anti-PPARγ and mouse anti-CD68. Sections were incubated with isotype control serum (Santa Cruz Biotechnology) or preimmune sera (AL-47) alone in place of primary antibody as a negative control or with the corresponding blocking peptide to identify the specificity of antibodies. After a 1-hour incubation at room temperature, sections were washed with PBS. Streptavidin biotin peroxidase or fluorescein isothiocyanate-conjugated goat anti-rabbit or tetramethylrhodamine B isothiocyanate-conjugated anti-mouse secondary antibody was added and incubated for 45 minutes at room temperature. Hematoxylin was used for nuclear counterstaining after peroxidase (diaminobenzidine) development, and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) was used for nuclear counterstaining after rhodamine incubation. In addition, differential interference contrast was used to identify the nuclear location of selected antigens. Images were captured using a Zeiss microscope and Axioviewer image analysis software (Deutsland; Carl Zeiss Corp., Jena, Germany).

Statistical Analysis

Results are presented as the mean ± SD. Data were analyzed using analysis of variance followed by Fisher's least significant difference test. P values <0.05 were considered significant.

Results

GHR Expression Is Reduced in CD Colon

Histological analysis (H&E staining and histological score) confirmed that the CD colon biopsies exhibited a moderate to severe degree of mucosal injury and inflammation, with a median CDHIS of 10 ($n = 8$; range, 4 to 14). The effect of active CD on colon GHR expression was not known. GHR was detected by immunohistochemistry (IHC) in both CECs and lamina propria mononuclear cells (LPMCs) of normal human colon. By comparison, IHC demonstrated a reduced frequency of GHR-positive CECs in affected areas of CD colon (Figure 1A). LCM was used to isolate CECs from biopsy sections, and total RNA was prepared. Real-time PCR demonstrated that GHR mRNA expression was significantly reduced in inflamed CECs, from 0.2 ± 0.1 to 0.012 ± 0.015 relative units (Figure 1B). Western blot using cytosolic proteins prepared from colon biopsies confirmed a significant reduction in GHR abundance in CD (Figure 1C). We then asked whether this led to a local reduction in steady-state and/or GH-dependent STAT5 activation.

STAT5b Activation Is Reduced in CD Colon

IHC confirmed that the frequency of tyrosine-phosphorylated STAT5 (pSTAT5)-positive CECs and LPMCs was reduced in CD colon, whereas nuclear p65 abundance was increased in the same sections (Figure 2A). Immunofluorescence (IF) with double labeling for pSTAT5 and CD68 identified a significant decrease in the frequency of pSTAT5 expressing CECs and $CD68^+$ lamina propria macrophages in CD colon (Figure 2B). We found that most subepithelial CD68-positive cells in normal colon were pSTAT5-positive; this was significantly reduced in inflamed colon.

We then asked whether GH-dependent activation of STAT5b would be impaired in CD colon. As shown in Figure 3A, we found that short-term exposure to GH (500 ng/ml for 45 minutes) activated STAT5b in normal colon, as demonstrated by increased nuclear extract binding to an IGF-1 gene promoter STAT5b *cis*-element on EMSA and increased frequency of pSTAT5-positive CECs and LPMCs in treated samples (Figure 3, A and B). By comparison, CD colon exhibited a significant resistance to GH stimulation of STAT5b activation. Overall STAT5b binding (relative to PBS-treated samples) was equal to 42.2 ± 17.6 relative units in normal colon after GH treatment compared with 7.9 ± 16.3 relative units in CD colon. Western blot for SHP1 confirmed that overall nuclear protein abundance was equal between the samples (Figure 3A). Supershift demonstrated that the shifted complex contained both STAT5a and STAT5b. We then asked whether expression of the tolerogenic GH:STAT5b target gene PPARγ was reduced under these conditions.

PPARγ Expression Is Reduced in CD Colon in CECs and LPMs

Total RNA was prepared from colon biopsies. Real-time PCR demonstrated that total PPARγ mRNA expression was significantly reduced in CD colon, from 1.8 ± 0.8 to 0.5 ± 0.2 relative units (Figure 4A). As shown, this included samples with moderate to severe degrees of histological inflammation. IHC showed that PPARγ abundance was reduced in both CECs and LPMCs (Figure 4B). IF with co-labeling for PPARγ and CD68 (as a marker for macrophages) showed that PPARγ expression was reduced in both CECs and subepithelial $CD68^+$ macrophages (Figure 4C). This was quite similar to the pattern of expression of pSTAT5 in these sections.

Taken together, these patient-based studies demonstrated a local reduction in CEC GHR expression in inflamed CD colon, leading to reduced GH-dependent STAT5b activation and PPARγ expression. Because STAT5a and STAT5b have previously been considered to exert a redundant effect with respect to mucosal tolerance, we then asked whether deficiency of STAT5b alone would lead to reduced PPARγ nuclear abundance and increased susceptibility to experimental colitis.

STAT5b Deficiency Increases Susceptibility to Colitis Due to TNBS Administration

Western blots using antibodies specific for STAT5a and STAT5b confirmed that there was no detectable STAT5b in the colon of STAT5b-deficient mice, relative to abundant expression of STAT5b in wild-type controls. STAT5a was present in low abundance in both wild-type and STAT5b-deficient colon (data not shown). With PBS enemas, STAT5b-deficient mice exhibited mild focal intracellular edema and rare focal neutrophilic infiltrates compared with wild-type (WT) controls. This led to a nonsignificant increase in the average histological score (Figure 5). These changes did not progress in older mice, up to 9 months of age. Relative to PBS, administration of 50% ethanol enemas led to a mild increase in endoscopic edema and mild focal histological erosions that did not differ between WT and STAT5b-deficient mice (Figure 5).

We then administered low-dose (1 mg) TNBS to STAT5b-deficient mice and WT controls and determined the effect on endoscopic, histological, and molecular markers of disease activity. WT mice exhibited erythema on endoscopy and mild inflammation on histology, which was greater in the distal colon than the proximal colon (Figure 5). Overall weight was reduced from 21.4 ± 1.2 to 20.7 ± 0.9 g. By day 4 after TNBS

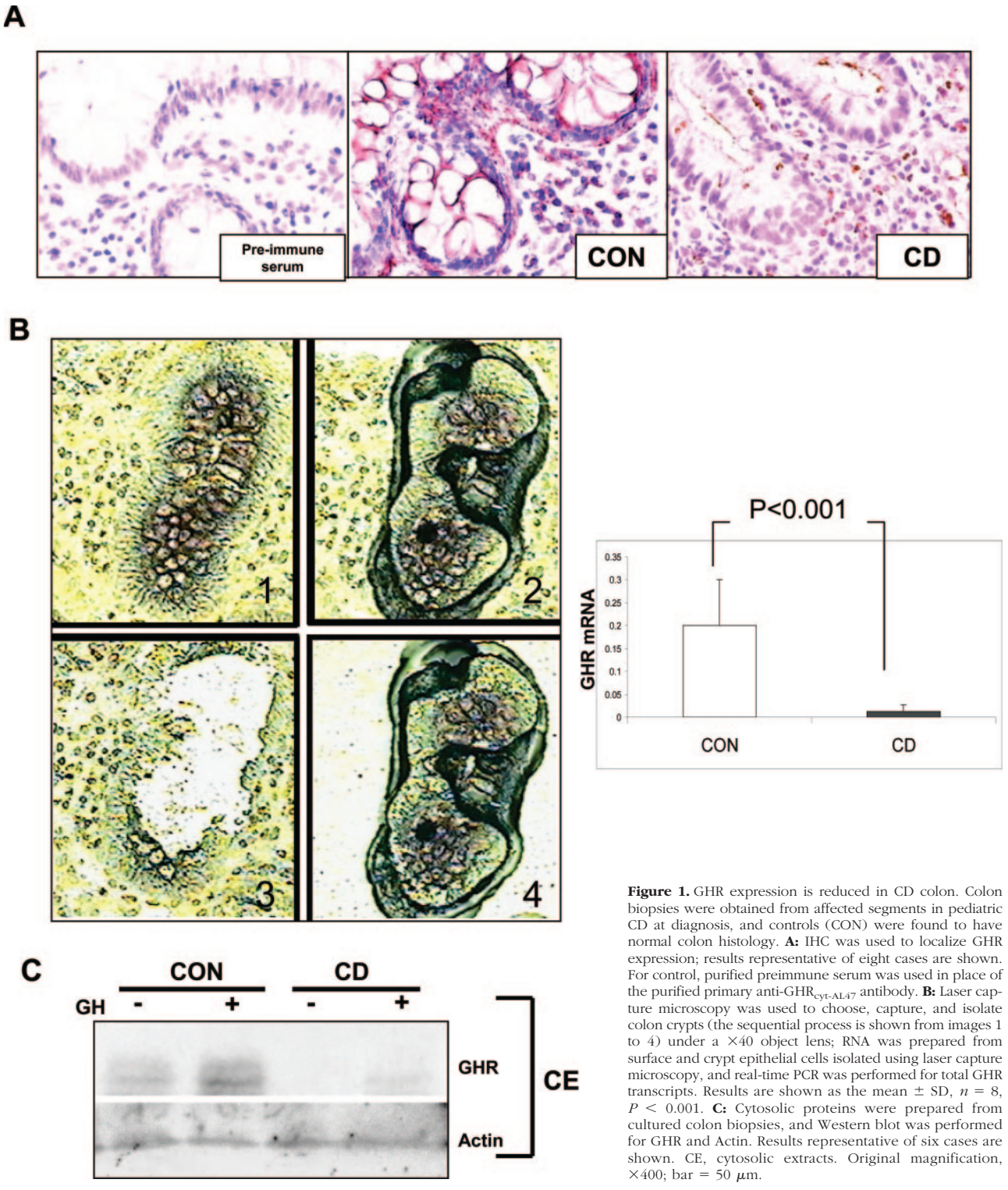
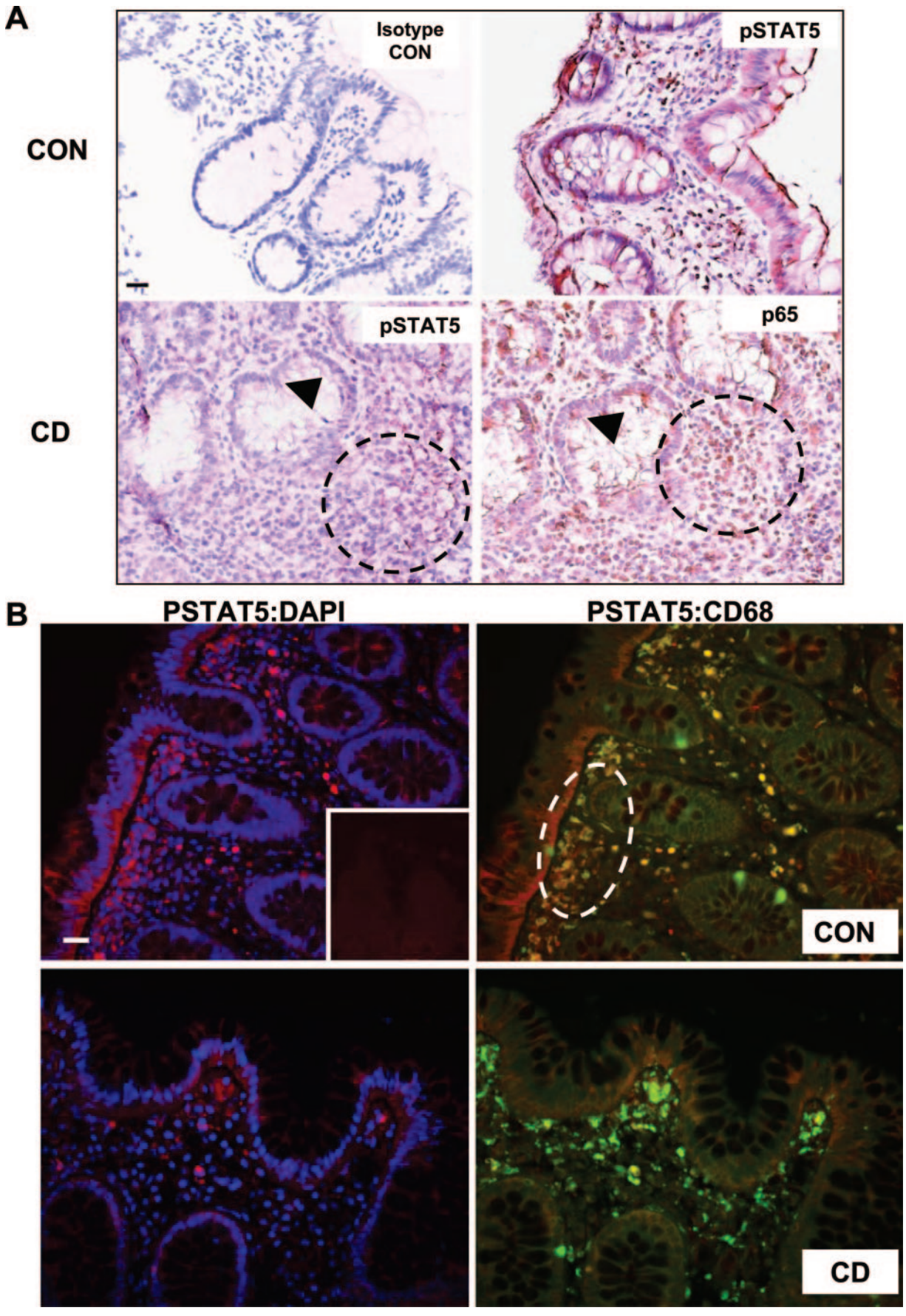


Figure 1. GHR expression is reduced in CD colon. Colon biopsies were obtained from affected segments in pediatric CD at diagnosis, and controls (CON) were found to have normal colon histology. **A:** IHC was used to localize GHR expression; results representative of eight cases are shown. For control, purified preimmune serum was used in place of the purified primary anti-$GHR_{cyt\text{-}AL47}$ antibody. **B:** Laser capture microscopy was used to choose, capture, and isolate colon crypts (the sequential process is shown from images 1 to 4) under a ×40 object lens; RNA was prepared from surface and crypt epithelial cells isolated using laser capture microscopy, and real-time PCR was performed for total GHR transcripts. Results are shown as the mean ± SD, $n = 8$, $P < 0.001$. **C:** Cytosolic proteins were prepared from cultured colon biopsies, and Western blot was performed for GHR and Actin. Results representative of six cases are shown. CE, cytosolic extracts. Original magnification, ×400; bar = 50 μm.

administration, STAT5b-deficient mice exhibited significantly greater mucosal injury on endoscopy (Figure 5A). This included more severe bleeding and ulceration and overall weight loss from 19.8 ± 1.0 to 18.3 ± 1.1 g. Histological analysis demonstrated increased epithelial injury and transmural inflammation in the STAT5b-deficient mice relative to the mild inflammation that developed in WT controls. Scoring of the lesions in the proximal versus distal colon demonstrated that the increased susceptibility to colitis in the STAT5b-deficient mice was confined to the proximal colon (Figure 5C). The histological score was 1.4 ± 0.7 in the wild-type controls in proximal colon, versus 2.9 ± 1.0 in the STAT5b-deficient mice, after TNBS administration ($P < 0.01$). There was no difference in the mild degree of inflammation that was observed in the distal colon.


A
CON
CD
Isotype CON
pSTAT5
pSTAT5
p65
B
PSTAT5:DAPI
PSTAT5:CD68
CON
CD

STAT5b Deficiency Reduces Colon PPARγ Abundance and Increases NF-κB Activation

We then determined whether PPARγ nuclear abundance was reduced in the STAT5b-deficient mice and whether this could be related to the increased susceptibility to proximal colitis. As shown in Figure 6A, we found that PPARγ nuclear abundance was significantly reduced in the proximal colon of PBS-treated STAT5b-deficient mice relative to the WT controls. Overall nuclear abundance of PPARγ was 18.4 ± 5.9 in the WT controls in the proximal colon, versus 5.5 ± 0.4 in the STAT5b-deficient mice. There was no detectable PPARγ by Western blot in colon cytosolic extracts in either STAT5b-deficient mice or WT controls (data not shown). PPARγ nuclear abundance was modestly reduced in the distal colon of STAT5b-deficient mice, although this was not statistically significant. IHC demonstrated a reduced frequency of PPARγ-positive proximal colon CECs and LPMCs in STAT5b-deficient mice relative to WT controls (Figure 6B).

Prior studies have shown that PPARγ may reduce NF-κB activation by associating with p65 in the nucleus and promoting shuttling of the PPARγ:p65 complex to the cytosol.[16] Immunoprecipitation of colon nuclear extracts with p65 followed by Western blot for PPARγ demonstrated that p65 was associated with PPARγ in the nucleus of both WT and STAT5b-deficient mice (Figure 6C). Interestingly, this was reduced from 27.2 ± 4 in the PBS-treated WT controls to 21.2 ± 2 in the PBS-treated STAT5b-deficient mice, with a further reduction in both genotypes after TNBS administration. Immunoprecipitation of colon nuclear extracts with PPARγ followed by Western blot for p65 confirmed that p65 was associated with PPARγ in the nucleus of both WT and STAT5b-deficient mice (Figure 6C). We did not observe association between PPARγ and the p50 NF-κB subunit under these conditions (data not shown). Taken together, these data showed that PPARγ and p65 were associated in the nucleus in the normal and inflamed colon, although the alterations in total nuclear abundance of PPARγ and p65 under these conditions prevented us from making any firm conclusions about differences in binding. We then asked whether the reduced PPARγ nuclear abundance in the STAT5b-deficient mice led to increased NF-κB activation.

As shown in Figure 7A, p65 nuclear abundance in proximal colon was increased significantly after TNBS administration to STAT5b-deficient mice, from 29.3 ± 3.6 to 54.6 ± 8.1. By comparison, TNBS administration did not lead to a significant increase in colon p65 nuclear abundance in the WT controls. Nuclear abundance of p65 did not differ between WT and STAT5b-deficient mice in the distal colon, with or without TNBS. NF-κB EMSA confirmed these results (Figure 7B). The signal for NF-κB binding was increased in both PBS- and TNBS-treated STAT5b-deficient mice, relative to WT controls. After TNBS administration, NF-κB-binding activity increased to 26.6 ± 5.3 in the WT controls, versus 50.1 ± 6.6 in the STAT5b-deficient mice. IHC for nuclear p65 localized the increase in NF-κB activation in the STAT5b-deficient mice to both the CECs and LPMCs in the more severely affected proximal colon (Figure 7C). Supershift confirmed that the majority of the shifted complex was composed of the p65 subunit. These data demonstrated that deficiency of STAT5b alone would increase susceptibility to experimental colitis, in conjunction with a reduction in local PPARγ abundance. We then asked whether the beneficial effect of PPARγ ligand pretreatment would be abrogated in STAT5b-deficient mice.

Rosiglitazone Pretreatment Ameliorates Colitis in WT but Not STAT5b-Deficient Mice

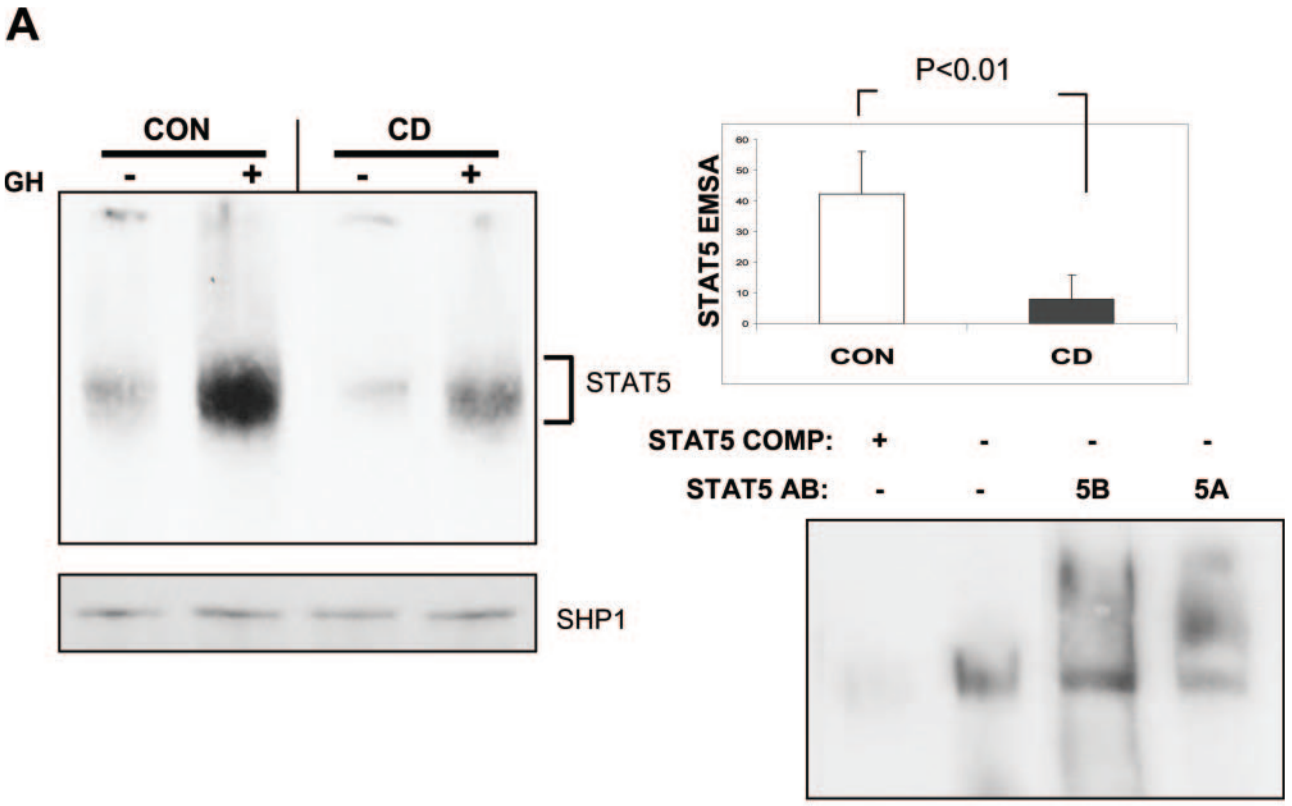
We provided rosiglitazone for 3 days before inducing colitis with 2 mg of TNBS and continued treatment until sacrifice on day 4. Rosiglitazone pretreatment reduced weight loss in WT mice from 6.8 ± 2.7 to 2.8 ± 3.9%. By comparison, weight loss increased in STAT5b-deficient mice with rosiglitazone pretreatment, from 5.3 ± 3 to 14.5 ± 2.2%. The higher dose of TNBS induced more significant distal colitis in the WT controls than had been observed with the lower dose [1 mg (Figure 5) versus 2 mg (Figure 8)]. Consistent with the effect on weight loss, rosiglitazone pretreatment significantly reduced histological disease activity in WT mice ($P < 0.05$) in both the distal and proximal colon but had no effect in STAT5b-deficient mice (Figure 8, A and B). Disease activity in the proximal colon remained higher in STAT5b-deficient mice compared with WT controls; this was more pronounced with rosiglitazone pretreatment. Consistent with these differences in histological severity, p65 nuclear abundance was reduced significantly by rosiglitazone pretreatment in WT mice ($P < 0.05$) and was not affected in STAT5b-deficient mice (Figure 8C).

Discussion

CD is becoming increasingly common in North America, with approximately 1,000,000 individuals affected.[37] Recent genetic studies have implicated primary defects in mucosal innate immunity that then lead to chronic, relapsing and remitting T-cell-predominant gut inflammation.[1] We asked whether the GH-dependent STAT5b transcription factor, which we have recently linked to growth failure in colitis, might also regulate mucosal inflammation.[30,38] In the current study, we have identified a novel anti-inflammatory STAT5b-dependent pathway in the colon in children with newly diagnosed CD and in murine colitis.

Targeted deletion of the GHR has recently identified PPARγ as a GH target gene, and GH has been shown to regulate the human PPARγ3 gene promoter via activation

Figure 2. Steady-state STAT5 activation is reduced in CD colon. **A:** IHC was used to localize expression of tyrosine pSTAT5 and nuclear p65. **Arrowheads** show reduced pSTAT5 and activated p65 in crypt epithelial cells; results representative of eight cases are shown. **B:** IF with labeling for pSTAT5 and CD68 was used to determine whether alterations in pSTAT5 localized to $CD68^+$ cells. pSTAT5, red; CD68, green; representative co-labeled cells are identified as circled, orange; nuclei were stained with DAPI, blue. The results for the isotype control are shown in the **inset** and included an isotype control IgG antibody in place of the primary pSTAT5 antibody. Results representative of eight cases are shown. Original magnification, ×400; bar = 50 μm.



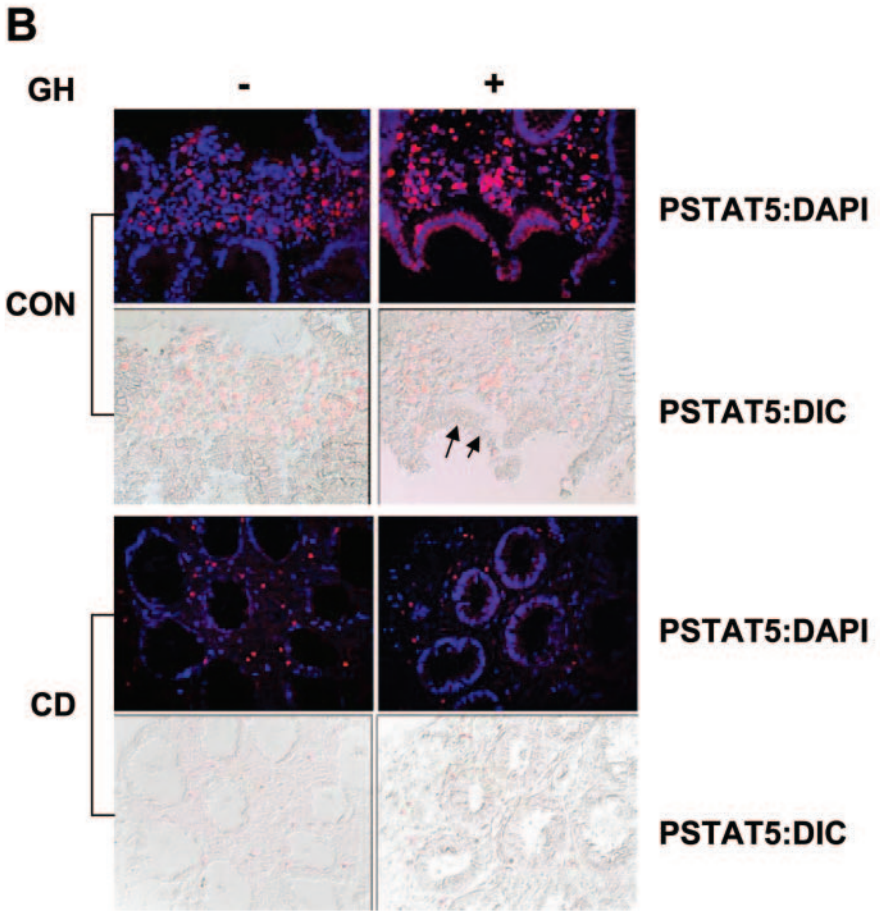


Figure 3. GH-dependent STAT5 activation is reduced in CD colon. CD and control colon biopsies were maintained in short-term organ culture and exposed to GH (500 ng/ml for 45 minutes) or PBS. **A:** Nuclear proteins were prepared and EMSA was performed using a STAT5b *cis*-element, and Western blot was performed using an SHP1 antibody. The signal intensity was quantified by densitometry for GH-treated control and CD samples and is shown as the mean ± SEM, $n = 8$. Supershift was performed using STAT5a and STAT5b antibodies. **B:** IF was used to localize alterations in pSTAT5 expression with GH treatment in CD versus controls. pSTAT5, red. DAPI, blue. Differential interference contrast was also used to identify cellular structures and nuclear localization of the pSTAT5 signal, as shown. Results representative of eight cases are shown. Original magnification, ×400; bar = 50 μm.

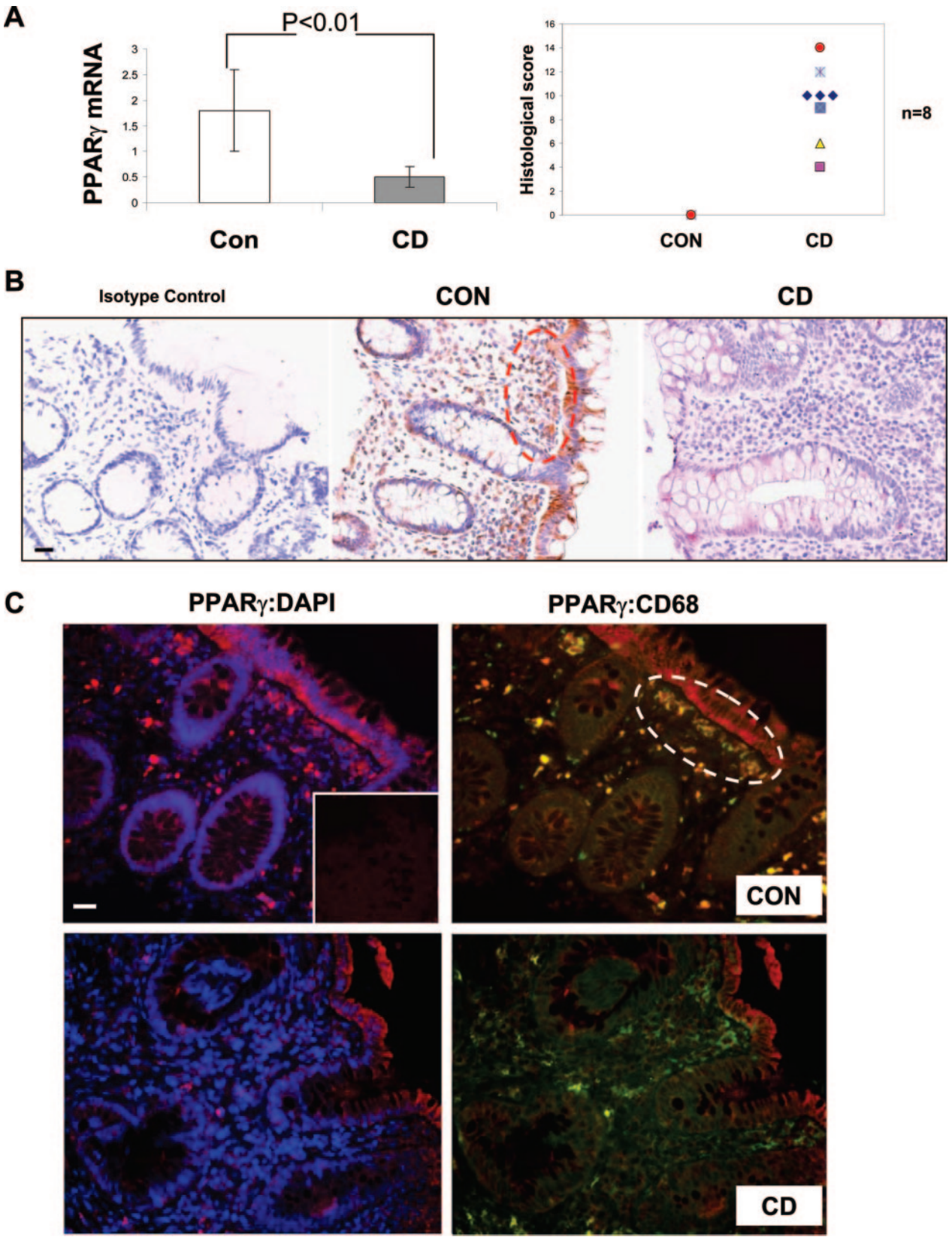


Figure 4. PPARγ expression is reduced in CD colon. **A:** RNA was prepared from control and CD colon biopsies, and real-time PCR was performed for total PPARγ transcripts. Results are shown as the mean ± SD, $n = 8$, $P < 0.01$. The CDHIS for each of the samples is shown. **B:** IHC was used to localize alterations in PPARγ; results representative of eight cases are shown. Isotype control included an isotype control IgG antibody in place of the primary PPARγ antibody. **C:** IF with labeling for PPARγ and CD68 was used to determine whether alterations in PPARγ localized to $CD68^+$ cells. PPARγ, red; CD68, green; representative co-labeled cells are identified are circled, orange; nuclei were stained with DAPI, blue. The results for the isotype control are shown in the **inset** and included an isotype control IgG antibody in place of the primary PPARγ antibody. Results representative of eight cases are shown. Original magnification, ×400; bar = 50 μm.

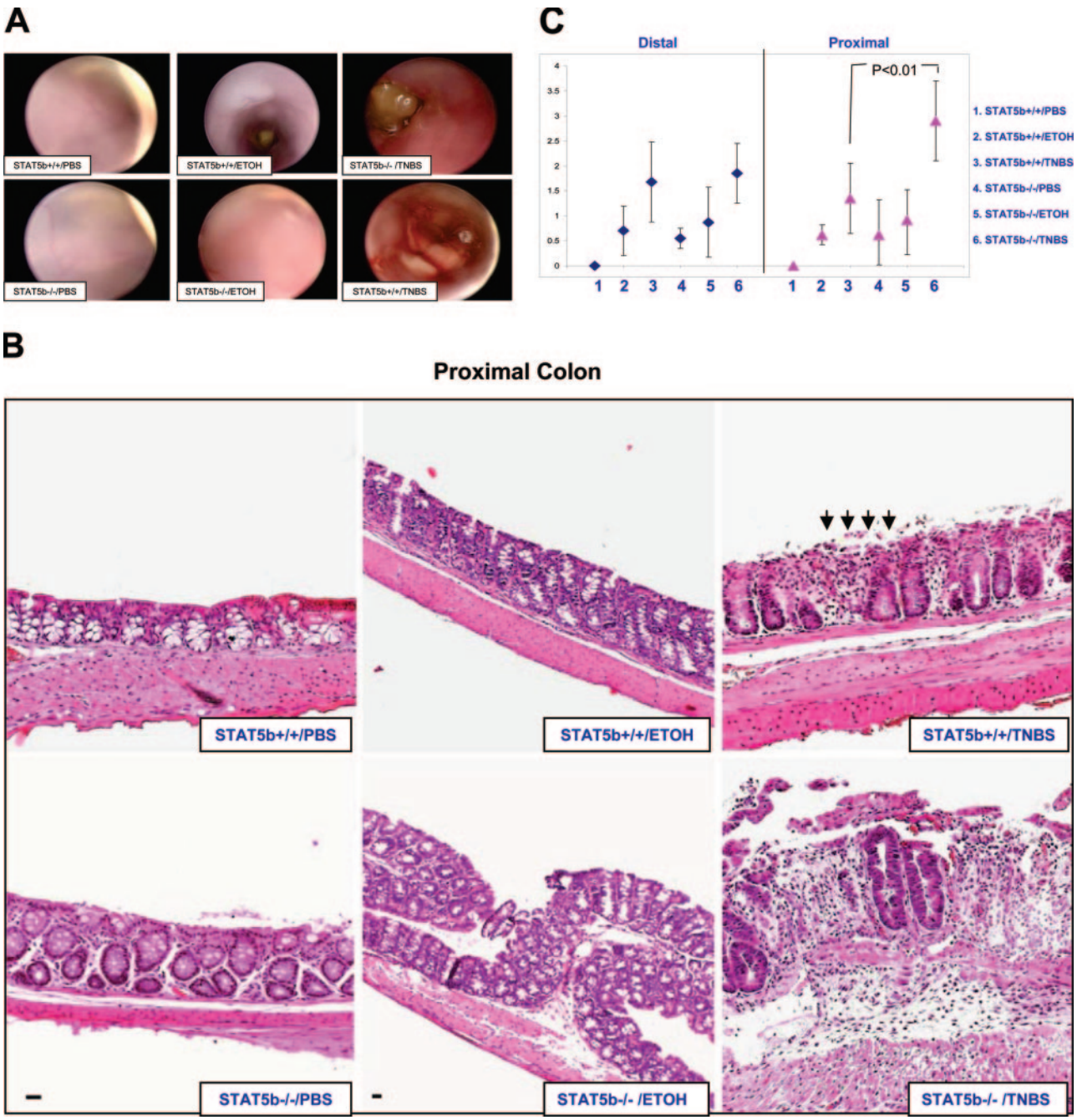


Figure 5. STAT5b deficiency increases the severity of proximal colitis as a result of TNBS administration. STAT5b-deficient mice and WT controls received PBS, ethanol, or TNBS enemas, and disease severity was assessed on day 4 by endoscopy (**A**), histology (**B**), and histological scores (**C**). In **A** and **B**, results representative of seven cases are shown. In **C**, the mean ± SD of the histological score in the distal and proximal colon is shown for each group as indicated, $n = 7$. Original magnification, ×400; bar = 50 μm.

of STAT5b.[25,39] The GHR is expressed on small bowel and colon epithelial cells and resident macrophages.[40] However, it was not known whether GH would activate STAT5b in normal human colon. Because we have recently shown that GH activation of STAT5b and IGF-1 is reduced in liver in murine colitis, we asked whether the GH:STAT5b:PPARγ pathway would similarly be reduced in inflamed colon.[30]

We found that GH-dependent STAT5b activation and PPARγ mRNA expression were significantly reduced in the affected colon of children with CD at diagnosis. IF identified a high constitutive expression of activated STAT5 and PPARγ in CECs and subepithelial LPMs of healthy controls, relative to a reduction in both cell populations in CD. Dubuquoy et al[22] also found that PPARγ RNA expression was reduced in adults with both CD and ulcerative colitis (UC). However, they identified a reduction at the protein level, which localized to CECs, only in patients with UC. Differences in the patient populations studied may account for this difference. We studied pa-

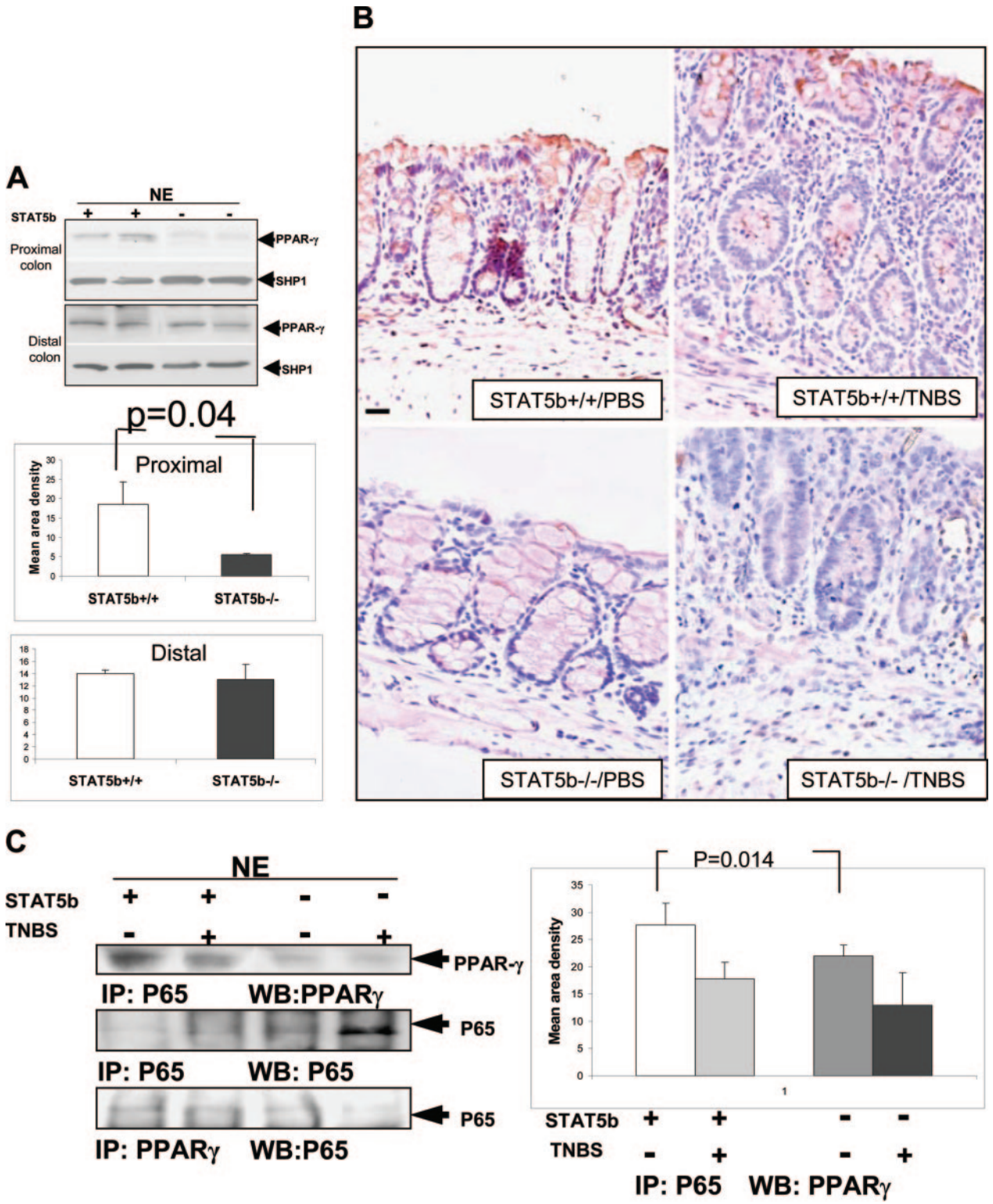


Figure 6. Proximal colon PPARγ nuclear abundance and PPARγ:p65 association were reduced in STAT5b-deficient mice. **A:** Western blots were performed for PPARγ and SHP1 using proximal and distal colon nuclear extracts (NE). The signal intensity was determined by densitometry and is shown as the mean ± SD, $n = 9$. **B:** IHC was used to localize alterations in PPARγ abundance. Results representative of seven cases are shown. **C:** Nuclear proteins were immunoprecipitated using anti-p65 or anti-PPARγ, and then Western blot was prepared and probed for PPARγ or p65, respectively. The signal intensity was determined by densitometry and is shown as the mean ± SD, $n = 7$. Original magnification, ×400; bar = 50 μm.

tients at diagnosis, before therapy, and the majority of the samples were obtained from the proximal colon, in the most affected segment. Therefore, differences in regulation of PPARγ protein abundance between proximal and distal colon and in response to therapy may account for the reduction in PPARγ identified in the present study.[41]

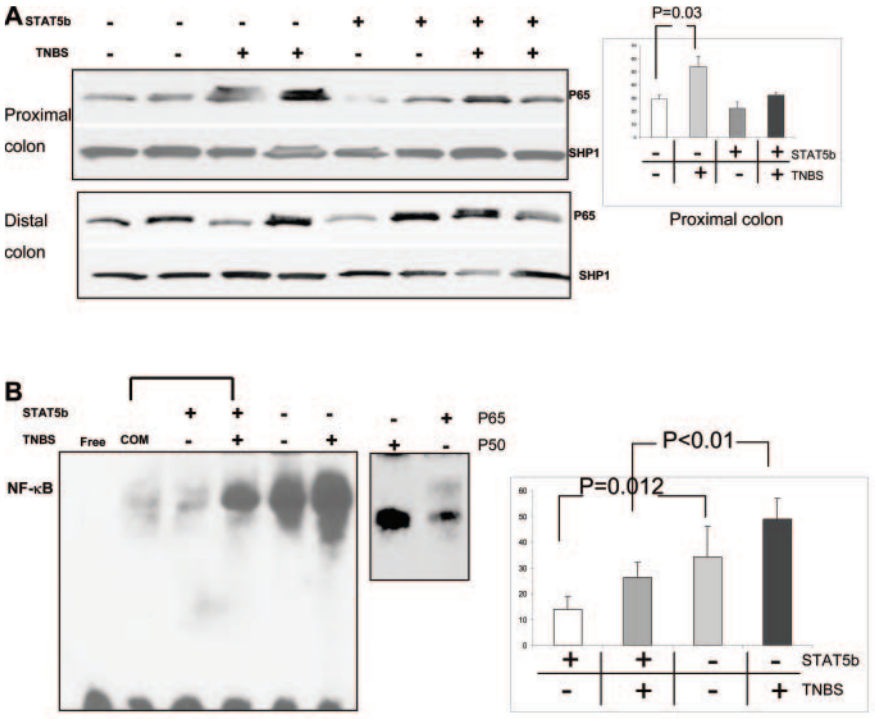


Figure 7. Colon NF-κB activation is increased in STAT5b-deficient mice. Proximal and distal colon nuclear proteins were made from STAT5b-deficient mice and WT controls, and Western blot for p65 and SHP1 (**A**) and EMSA for a NF-κB *cis*-element with supershift for p65 and p50 (**B**) were performed. Signal intensity was determined by densitometry and is shown as the mean ± SD, $n = 7$. **C:** IHC was performed to localize alterations in p65 nuclear abundance. Results representative of seven cases are shown. Original magnification, ×400; bar = 50 μm.

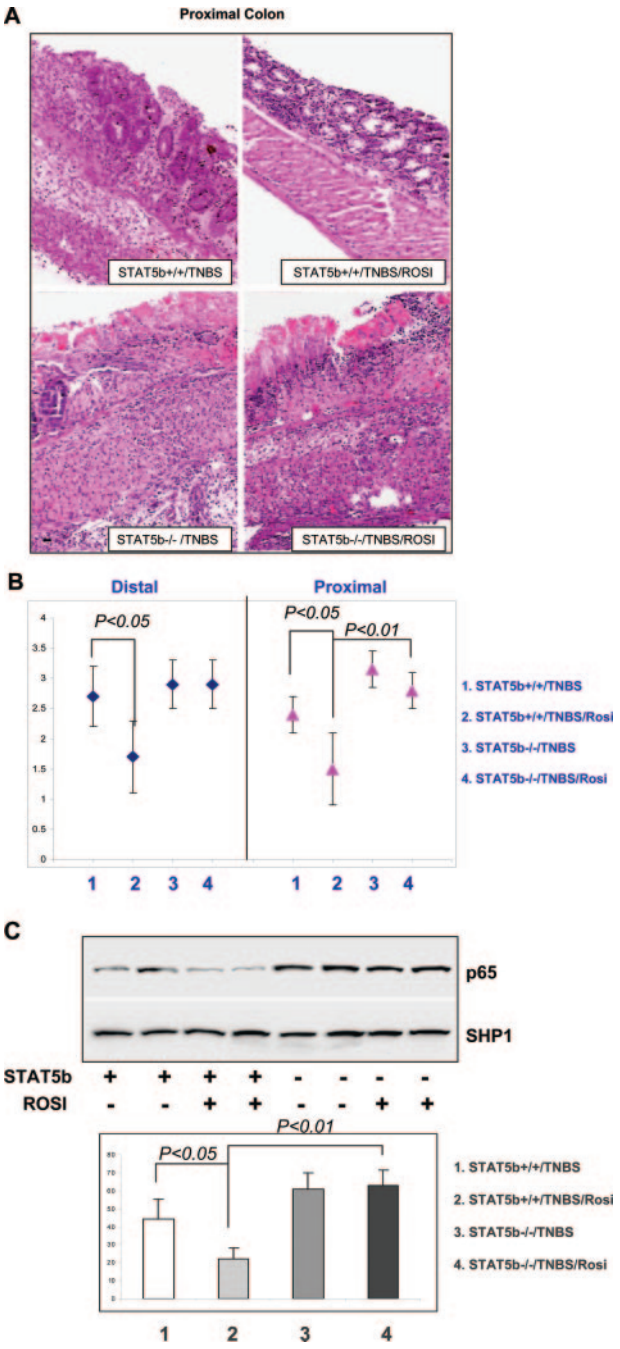


Figure 8. Rosiglitazone pretreatment ameliorates colitis in WT but not STAT5b-deficient mice. WT and STAT5b-deficient mice received rosiglitazone (Rosi) for 3 days before induction of TNBS colitis and continued treatment until sacrifice. **A:** Representative images from proximal colon are shown. **B:** The mean ± SD of the histological score in the distal and proximal colon is shown for each group as indicated, $n = 4$ or 5. **C:** Proximal colon nuclear proteins were prepared, and p65 and SHP1 abundances were determined by Western blot. Signal intensity was determined by densitometry and is shown as the mean ± SD, $n = 4–5$.

Costello et al[3] have recently reported that STAT5b mRNA expression is reduced in affected colon of adults with CD and UC. Therefore, it is likely that this pathway contributes to chronic mucosal inflammation in both Crohn's colitis and UC and may be applicable to both adults and children with inflammatory bowel disease. We are currently examining the GH:STAT5b:PPARγ signaling pathway using samples from children with UC at diagnosis. It is important to note that Hommes and colleagues[42] have recently reported in a letter to the editors an increase in activated STAT5 in the colon of adults with steroid-resistant ulcerative colitis, and we have found that STAT5 is activated in LPMCs of mice with colitis due to interleukin (IL)-10 deficiency.[43] Therefore, cellular STAT5 abundance and activation may vary between different types of human and murine colitis.

It was not known what the functional significance of a deficiency in STAT5b alone would be with respect to susceptibility to colitis. We therefore determined whether STAT5b-deficient mice were more susceptible to colitis due to TNBS administration. We found that PPARγ nuclear abundance was reduced in the proximal but not distal colon of STAT5b-deficient mice and that this led to increased NF-κB activation and more severe proximal colitis after TNBS administration. These data have identified STAT5b for the first time as a critical regulator of colonic mucosal tolerance and may provide insight into the regional differences in gut inflammation that are observed in patients with CD.

It is important to note that Shipley and Waxman[44] have previously shown that GH-activated STAT5b can inhibit expression of ligand-activated PPARγ target genes, and vice versa, via a bi-directional posttranslational negative feedback loop. However, Rieusset et al[45] have reported that male PPARγ$^{+/-}$ mice have reduced linear growth and weight gain and have shown that GH signaling is actually impaired in white adipose tissue of these mice, due to up-regulation of SOCS-2. Our studies indicate that GH-activated STAT5b positively regulates PPARγ nuclear protein abundance in the colon. In fact, we have recently reported that chronic GH administration will activate STAT5b and up-regulate PPARγ expression in normal and inflamed mouse colon.[43] This may be distinct from effects of GH-dependent STAT5b activation on posttranslational regulation of PPARγ-dependent gene transcription by PPARγ ligands. Our data are consistent with recent work from Masternak et al,[46] demonstrating reduced skeletal muscle PPARγ protein abundance in mice with targeted deletion of the growth hormone receptor. However, this is in contrast to data from patients with acromegaly, in which chronic exposure to increased GH secretion and circulating IGF-1 is associated with increased CEC proliferation and reduced PPARγ expression, perhaps due to a direct effect of IGF-1 on CEC differentiation and gene expression.[47] Therefore, interactions between activated STAT5b and PPARγ are quite complex, and future studies will carefully determine the relative effects of GH and rosiglitazone administration on expression of STAT5b and PPARγ target genes in the normal and inflamed colon.

To determine more directly the functional significance of the reduction in proximal colon PPARγ abundance, we pretreated WT and STAT5b-deficient mice with the PPARγ agonist rosiglitazone for 3 days before administration of TNBS. PPARγ agonists have previously been shown to reduce the severity of colitis due to both TNBS and DSS administration and IL-10 deficiency.[15,18,19,24,48] These have been more consistently effective when administered before the onset of colitis, perhaps because of down-regulation of PPARγ abundance with the onset of colitis. We found that

rosiglitazone pretreatment reduced NF-κB activation and histological disease severity in WT but not STAT5b-deficient mice, supporting the functional importance of the reduction in PPARγ abundance. Interestingly, the beneficial effect of rosiglitazone was abrogated in both the proximal and distal colon of STAT5b-deficient mice, although a significant reduction in whole-colon PPARγ nuclear protein abundance was only observed in the proximal colon. A recent report has indicated that rosiglitazone may act primarily through nonepithelial cells to suppress colonic inflammation, because a beneficial effect of rosiglitazone was observed in DSS colitis in mice with targeted deletion of PPARγ in CECs.[49] Future studies using bone marrow chimeras will determine whether the requirement for STAT5b in mediating anti-inflammatory effects of rosiglitazone is in fact confined to a limited bone marrow-derived compartment, such as the subepithelial macrophages.

Patients with CD also frequently exhibit more severe inflammation in the proximal colon. Recently, Kim et al[50] have shown that mono-association with specific bacterial species will induce either proximal or distal colitis in mice with IL-10 deficiency. Several lines of evidence have indicated that expression of PPARγ in the colon is regulated by the enteric flora.[22] It would seem likely that the composition and adherence of the local enteric flora to the epithelium in the proximal colon may interact with local STAT5b activation and the mucosal barrier to regulate PPARγ nuclear abundance and susceptibility to colitis.[51,52] Loss of STAT5b may then disrupt this inherent tolerogenic mechanism, leading to colitis. This is being tested in ongoing studies.

It has not previously been proposed that a defect in STAT5b activation may contribute to both systemic growth failure and impaired mucosal tolerance in CD. We have now shown that a novel anti-inflammatory GH:STAT5b:PPARγ pathway is down-regulated in the inflamed colon in pediatric CD at diagnosis. Mice with STAT5b deficiency exhibit reduced PPARγ expression and increased susceptibility to proximal colitis after TNBS administration. It is important to note that GH and granulocyte-macrophage colony-stimulating factor, which can both directly activate STAT5b in CECs and LPMs, have also shown beneficial effects in early clinical and pre-clinical studies in Crohn's disease.[10,13,14,53,54] Therefore, augmenting intestinal STAT5b activation may represent a novel approach for restoring mucosal tolerance in inflammatory bowel disease.

Acknowledgments

Erin Bonkowski provided outstanding technical assistance. Edgar Ballard, M.D., assessed the CD histology. The STAT5b-null mice were provided by Dr. James Ihle (St. Jude Children's Research Hospital, Memphis, TN). Mouse colon sections for histological analysis were prepared in the Integrative Morphology Core of the National Institutes of Health-supported Children's Hospital Research Foundation Digestive Diseases Research and Development Center (grant R24 DK64403).

References

1. Bouma G, Strober W: The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003, 3:521–533
2. Neurath MF, Pettersson S, Meyer zum Buschenfelde KH, Strober W: Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice. Nat Med 1996, 2:998–1004
3. Costello CM, Mah N, Hasler R, Rosenstiel P, Waetzig GH, Hahn A, Lu T, Gurbuz Y, Nikolaus S, Albrecht M, Hampe J, Lucius R, Kloppel G, Eickhoff H, Lehrach H, Lengauer T, Schreiber S: Dissection of the inflammatory bowel disease transcriptome using genome-wide cDNA microarrays. PLoS Med 2005, 2:e199
4. Snow JW, Abraham N, Ma MC, Herndier BG, Pastuszak AW, Goldsmith MA: Loss of tolerance and autoimmunity affecting multiple organs in STAT5A/5B-deficient mice. J Immunol 2003, 171:5042–5050
5. Davey HW, Park SH, Grattan DR, McLachlan MJ, Waxman DJ: STAT5b-deficient mice are growth hormone pulse-resistant. Role of STAT5b in sex-specific liver p450 expression. J Biol Chem 1999, 274:35331–35336
6. Davey HW, Wilkins RJ, Waxman DJ: STAT5 signaling in sexually dimorphic gene expression and growth patterns. Am J Hum Genet 1999, 65:959–965
7. Davey HW, Xie T, McLachlan MJ, Wilkins RJ, Waxman DJ, Grattan DR: STAT5b is required for GH-induced liver IGF-I gene expression. Endocrinology 2001, 142:3836–3841
8. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN: Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 1998, 93:841–850
9. Chen K, Nezu R, Inoue M, Wasa M, Iiboshi Y, Fukuzawa M, Kamata S, Takagi Y, Okada A: Beneficial effects of growth hormone combined with parenteral nutrition in the management of inflammatory bowel disease: an experimental study. Surgery 1997, 121:212–218
10. Han X, Sosnowska D, Bonkowski E, Denson L: Growth hormone reduces STAT3 activation and improves disease activity in murine colitis. Gastroenterology 2005, 129:185–203
11. Kara E, Sungurtekin H, Sungurtekin U, Alkanat M, Ilkgul O: The effect of recombinant human growth hormone (rhGH) on trinitrobenzene sulfonic acid-induced colitis in rats: an experimental study. Inflamm Bowel Dis 2004, 10:112–115
12. Williams KL, Fuller CR, Dieleman LA, DaCosta CM, Haldeman KM, Sartor RB, Lund PK: Enhanced survival and mucosal repair after dextran sodium sulfate- induced colitis in transgenic mice that overexpress growth hormone. Gastroenterology 2001, 120:925–937
13. Theiss AL, Fuller CR, Simmons JG, Liu B, Sartor RB, Lund PK: Growth hormone reduces the severity of fibrosis associated with chronic intestinal inflammation. Gastroenterology 2005, 129:204–219
14. Slonim AE, Bulone L, Damore MB, Goldberg T, Wingertzahn MA, McKinley MJ: A preliminary study of growth hormone therapy for Crohn's disease. N Engl J Med 2000, 342:1633–1637
15. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, Schoonjans K, Derijard B, Desvergne B, Wahli W, Chambon P, Leibowitz MD, Colombel JF, Auwerx J: Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. J Exp Med 2001, 193:827–838
16. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S: Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 2004, 5:104–112
17. Katayama K, Wada K, Nakajima A, Mizuguchi H, Hayakawa T, Nakagawa S, Kadowaki T, Nagai R, Kamisaki Y, Blumberg RS, Mayumi T: A novel PPAR gamma gene therapy to control inflammation associated with inflammatory bowel disease in a murine model. Gastroenterology 2003, 124:1315–1324
18. Lytle C, Tod TJ, Vo KT, Lee JW, Atkinson RD, Straus DS: 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, 11:231–243
19. Schaefer KL, Denevich S, Ma C, Cooley SR, Nakajima A, Wada K, Schlezinger J, Sherr D, Saubermann LJ: Intestinal antiinflammatory effects of thiazolidenedione peroxisome proliferator-activated receptor-gamma ligands on T helper type 1 chemokine regulation include nontranscriptional control mechanisms. Inflamm Bowel Dis 2005, 11:244–252

20. Lewis JD, Lichtenstein GR, Stein RB, Deren JJ, Judge TA, Fogt F, Furth EE, Demissie EJ, Hurd LB, Su CG, Keilbaugh SA, Lazar MA, Wu GD: An open-label trial of the PPAR-gamma ligand rosiglitazone for active ulcerative colitis. Am J Gastroenterol 2001, 96:3323–3328
21. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD: A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999, 104:383–389
22. Dubuquoy L, Jansson EA, Deeb S, Rakotobe S, Karoui M, Colombel JF, Auwerx J, Pettersson S, Desreumaux P: Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. Gastroenterology 2003, 124:1265–1276
23. Rousseaux C, Lefebvre B, Dubuquoy L, Lefebvre P, Romano O, Auwerx J, Metzger D, Wahli W, Desvergne B, Naccari GC, Chavatte P, Farce A, Bulois P, Cortot A, Colombel JF, Desreumaux P: Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. J Exp Med 2005, 201:1205–1215
24. Saubermann LJ, Nakajima A, Wada K, Zhao S, Terauchi Y, Kadowaki T, Aburatani H, Matsuhashi N, Nagai R, Blumberg RS: Peroxisome proliferator-activated receptor gamma agonist ligands stimulate a Th2 cytokine response and prevent acute colitis. Inflamm Bowel Dis 2002, 8:330–339
25. Meirhaeghe A, Fajas L, Gouilleux F, Cottel D, Helbecque N, Auwerx J, Amouyel P: A functional polymorphism in a STAT5B site of the human PPAR gamma 3 gene promoter affects height and lipid metabolism in a French population. Arterioscler Thromb Vasc Biol 2003, 23:289–294
26. Cowan JW, Wang X, Guan R, He K, Jiang J, Baumann G, Black RA, Wolfe MS, Frank SJ: Growth hormone receptor is a target for presenilin-dependent gamma-secretase cleavage. J Biol Chem 2005, 280:19331–19342
27. D'Haens GR, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P: Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998, 114:262–267
28. D'haens G, Van Deventer S, Van Hogezand R, Chalmers D, Kothe C, Baert F, Braakman T, Schaible T, Geboes K, Rutgeerts P: Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial. Gastroenterology 1999, 116:1029–1034
29. Chow JY, Carlstrom K, Barrett KE: Growth hormone reduces chloride secretion in human colonic epithelial cells via EGF receptor and extracellular regulated kinase. Gastroenterology 2003, 125:1114–1124
30. DiFedele LM, He J, Bonkowski EL, Han X, Held MA, Bohan A, Menon RK, Denson LA: Tumor necrosis factor alpha blockade restores growth hormone signaling in murine colitis. Gastroenterology 2005, 128:1278–1291
31. Elson CO, Beagley KW, Sharmanov AT, Fujihashi K, Kiyono H, Tennyson GS, Cong Y, Black CA, Ridwan BW, McGhee JR: Hapten-induced model of murine inflammatory bowel disease: mucosa immune responses and protection by tolerance. J Immunol 1996, 157:2174–2185
32. Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W: Antibodies to interleukin 12 abrogate established experimental colitis in mice. J Exp Med 1995, 182:1281–1290
33. Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, Burg J, Strand S, Kiesslich R, Huber S, Ito H, Nishimoto N, Yoshizaki K, Kishimoto T, Galle PR, Blessing M, Rose-John S, Neurath MF: TGF-beta suppresses tumor progression in Th2 colitis and associated colon cancer by inhibition of IL-6 trans-signaling. Immunity 2004, 21:491–501
34. Kola B, Korbonits M, Diaz-Cano S, Kaltsas G, Morris DG, Jordan S, Metherell L, Powell M, Czirjak S, Arnaldi G, Bustin S, Boscaro M, Mantero F, Grossman AB: Reduced expression of the growth hormone and type 1 insulin-like growth factor receptors in human somatotroph tumours and an analysis of possible mutations of the growth hormone receptor. Clin Endocrinol (Oxf) 2003, 59:328–338
35. Woelfle J, Chia DJ, Rotwein P: Mechanisms of growth hormone (GH) action: identification of conserved Stat5 binding sites that mediate GH-induced insulin-like growth factor-I gene activation. J Biol Chem 2003, 278:51261–51266
36. Han X, Fink MP, Uchiyama T, Yang R, Delude RL: Increased iNOS activity is essential for pulmonary epithelial tight junction dysfunction in endotoxemic mice. Am J Physiol Lung Cell Mol Physiol 2004, 286:L259–L267
37. Kugathasan S, Judd RH, Hoffmann RG, Heikenen J, Telega G, Khan F, Weisdorf-Schindele S, San Pablo Jr W, Perrault J, Park R, Yaffe M, Brown C, Rivera-Bennett MT, Halabi I, Martinez A, Blank E, Werlin SL, Rudolph CD, Binion DG: Epidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: a statewide population-based study. J Pediatr 2003, 143:525–531
38. Denson LA, Menon RK, Shaufl A, Bajwa HS, Williams CR, Karpen SJ: TNF-alpha downregulates murine hepatic growth hormone receptor expression by inhibiting Sp1 and Sp3 binding. J Clin Invest 2001, 107:1451–1458
39. Rowland JE, Lichanska AM, Kerr LM, White M, d'Aniello EM, Maher SL, Brown R, Teasdale RD, Noakes PG, Waters MJ: In vivo analysis of growth hormone receptor signaling domains and their associated transcripts. Mol Cell Biol 2005, 25:66–77
40. Lobie PE, Breipohl W, Waters MJ: Growth hormone receptor expression in the rat gastrointestinal tract. Endocrinology 1990, 126:299–306
41. Lefebvre M, Paulweber B, Fajas L, Woods J, McCrary C, Colombel JF, Najib J, Fruchart JC, Datz C, Vidal H, Desreumaux P, Auwerx J: Peroxisome proliferator-activated receptor gamma is induced during differentiation of colon epithelium cells. J Endocrinol 1999, 162:331–340
42. Löwenberg M, Scheffer M, Verhaar A, Peppelenbosch M, Hommes D: A role for STAT5 in steroid-resistant UC? Inflamm Bowel Dis 2006, 12:665
43. Han X, Benight N, Osuntokun B, Loesch K, Frank S, Denson L: Tumour necrosis factor {alpha} blockade induces an anti-inflammatory growth hormone signalling pathway in experimental colitis. Gut 2006, (in press)
44. Shipley JM, Waxman DJ: Simultaneous, bidirectional inhibitory crosstalk between PPAR and STAT5b. Toxicol Appl Pharmacol 2004, 199:275–284
45. Rieusset J, Seydoux J, Anghel SI, Escher P, Michalik L, Soon Tan N, Metzger D, Chambon P, Wahli W, Desvergne B: Altered growth in male peroxisome proliferator-activated receptor gamma (PPARgamma) heterozygous mice: involvement of PPARgamma in a negative feedback regulation of growth hormone action. Mol Endocrinol 2004, 18:2363–2377
46. Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, Bonkowski MS, Panici JA, Przybylski GK, Bartke A: Caloric restriction results in decreased expression of peroxisome proliferator-activated receptor superfamily in muscle of normal and long-lived growth hormone receptor/binding protein knockout mice. J Gerontol A Biol Sci Med Sci 2005, 60:1238–1245
47. Bogazzi F, Ultimieri F, Raggi F, Costa A, Gasperi M, Cecconi E, Mosca F, Bartalena L, Martino E: Peroxisome proliferator activated receptor gamma expression is reduced in the colonic mucosa of acromegalic patients. J Clin Endocrinol Metab 2002, 87:2403–2406
48. Sánchez-Hidalgo M, Martin AR, Villegas I, Alarcon De La Lastra C: Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, reduces chronic colonic inflammation in rats. Biochem Pharmacol 2005, 69:1733–1744
49. Adachi M, Kurotani R, Morimura K, Shah Y, Sanford M, Madison BB, Gumucio DL, Marin HE, Peters JM, Young HA, Gonzalez FJ: PPAR{gamma} in colonic epithelial cells protects against experimental inflammatory bowel disease. Gut 2006, 55:1104–1113
50. Kim SC, Tonkonogy SL, Albright CA, Tsang J, Balish EJ, Braun J, Huycke MM, Sartor RB: Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology 2005, 128:891–906
51. Swidsinski A, Loening-Baucke V, Lochs H, Hale LP: Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. World J Gastroenterol 2005, 11:1131–1140
52. Swidsinski A, Weber J, Loening-Baucke V, Hale LP, Lochs H: Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J Clin Microbiol 2005, 43:3380–3389
53. Lehtonen A, Matikainen S, Miettinen M, Julkunen I: Granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced STAT5 activation and target-gene expression during human monocyte/macrophage differentiation. J Leukoc Biol 2002, 71:511–519
54. Korzenik JR, Dieckgraefe BK, Valentine JF, Hausman DF, Gilbert MJ: Sargramostim for active Crohn's disease. N Engl J Med 2005, 352:2193–2201