Human Intestinal Epithelial Cells Express Interleukin-10 through Toll-Like Receptor 4-Mediated Epithelial-Macrophage Crosstalk

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Key Words

Epithelial cells · Interleukin-10 · Intestine · Macrophages · Peroxisome proliferator-activated receptor · Toll-like receptors

Abstract

In the intestine, interaction between epithelial cells and macrophages (MΦs) create a unique immunoregulatory microenvironment necessary to maintain local immune and tissue homeostasis. Human intestinal epithelial cells (IECs) have been shown to express interleukin (IL)-10, which keeps epithelial integrity. We have demonstrated that bacterial signaling through Toll-like receptor (TLR) 4 induces 15-deoxy-Δ^{12,14}-prostaglandin J₂ (15d-PGJ₂) synthesis in intestinal MΦs by cyclooxygenase (Cox)-2 expression. Here, we show that TLR4 signaling generates crosstalk between IECs and MΦs that enhances IL-10 expression in IECs. Direct stimulation of TLR4 leads to the expression of IL-10 in IECs, while the presence of MΦs in a Transwell system induces another peak in IL-10 expression in IECs at a later time point. The second peak of the IL-10 expression is two times greater than the first peak. This late induction of IL-10 depends on the nuclear receptor peroxisome proliferator-activated receptor

(PPAR) γ that is accumulated in IECs by TLR4-mediated inhibition of the ubiquitin-proteasomal pathway. TLR4 signaling in MΦs in turn synthesizes 15d-PGJ₂ through p38 and ERK activation and Cox-2 induction, which activates PPARγ in IECs. These results suggest that TLR4 signaling maintains IL-10 production in IECs by generating epithelial-MΦs crosstalk, which is an important mechanism in the maintenance of intestinal homeostasis mediated through host-bacterial interactions.

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Introduction

Intestinal mucosal immune responses to commensal bacteria are continuously controlled by endogenous immunoregulatory mechanisms. As an interface between host and commensal bacteria, intestinal epithelial cells (IECs) play crucial roles in keeping mucosal homeostasis largely through the regulation of innate immunity. The loss of this homeostasis results in chronic inflammation of mucosa seen in inflammatory bowel diseases (IBDs). Toll-like receptors (TLRs) are major innate immune receptors that induce cellular and molecular signaling path-

ways to maintain intestinal epithelial integrity by recognizing pathogen-associated molecular patterns of luminal microbes [1, 2]. This response is particularly important when the mucosal barrier is disrupted following exposure of the basolateral aspect of IECs and lamina propria immune cells to microbial products where TLRs may be activated [3]. Leakage of the epithelial barrier is known to occur frequently in intestinal mucosa even upon psychological stress and predisposes to irritable bowel diseases and IBD [4, 5]. While the inflammatory responses that may be triggered against commensal flora are regulated in healthy intestinal mucosa, underlying mechanisms of the regulation remain to be fully understood.

Interleukin 10 (IL-10) is one of the major anti-inflammatory cytokines required to control host immune responses to intestinal bacteria since IL-10-deficient mice develop spontaneous colitis only in the presence of commensal bacteria [6, 7]. IL-10 has not only been shown to suppress proinflammatory signaling, but also to maintain intestinal epithelial barrier function [8, 9]. Although IL-10 reporter knockin mice have demonstrated the absence of IL-10 expression in IECs, IECs have been identified as the major cell type expressing IL-10 in normal human intestine [10, 11]. This expression of IL-10 by IECs appears to be physiologically indispensable for epithelial homeostasis. This conclusion is supported by the fact that blocking IL-10 production by IECs leads to a disruption of the epithelial lining in the human intestine due to excessive apoptosis and IFN- γ production [9]. Interestingly, the production of IL-10 from IECs is not observed in germ-free rats, but it is induced after colonization of commensal bacteria [12]. Therefore, bacterial signaling is likely involved in the homeostatic IL-10 production in IECs. Moreover, oral administration of chitosan, a type of oligosaccharide that acts as TLR4 ligand to rats, has been shown to induce IL-10 from IECs [13]. These findings suggest that IL-10 production from human IECs may be regulated by mucosal TLR4 signaling in response to luminal commensal bacteria.

We have demonstrated that TLR4 signaling regulates anti-inflammatory 15-deoxy- Δ -12,14-prostaglandin J2 (15d-PGJ2) synthesis in the intestine via cyclooxygenase-2 (Cox-2) induction in subepithelial macrophages (M Φ s) [14]. The significance of TLR signaling-dependent Cox-2 induction in subepithelial M Φ s in IEC homeostasis has been reported in a mouse mucosal injury model [15, 16], but whether this regulation of IEC homeostasis is involved in IL-10 production from IECs has not been studied. 15d-PGJ2 is an endogenous ligand of the nuclear receptor peroxisome proliferator-activated receptor (PPAR) γ , which

possesses anti-inflammatory properties known to antagonize NF- κ B, AP-1 and STAT1 [17–19]. PPAR γ can be expressed by many cell types in humans, but adipocytes and IECs are known to be the two major cell types that abundantly express PPAR γ [20]. It has been shown that exposure of human IECs to *Enterococcus faecalis* isolated from the human intestine results in the activation of PPAR γ and IL-10 production in IECs [21]. Furthermore, TLR4 signaling may induce PPAR γ expression in IECs [22, 23]. These data suggest that epithelial PPAR γ integrates mucosal TLR4 signaling to IL-10 production in IECs.

In this study, we examined TLR4-mediated crosstalk between IEC and M Φ s that induces IL-10 production in IECs via PPAR γ activation. Co-culturing of polarized IECs with M Φ s resulted in late but enhanced IL-10 production by IECs in response to TLR4 stimulation. This late induction of IL-10 in IECs was not observed without M Φ TLR4 signaling. The crosstalk between M Φ s and IECs was mediated by the induction of 15d-PGJ2 synthesis in M Φ s. On the other hand, PPAR γ expression, nuclear accumulation and DNA binding activities in IECs were observed prior to the enhanced IL-10 production. Blocking PPAR γ expression or activation in IECs completely abolished the late IL-10 production. Moreover, we detected a PPAR γ binding site in the human IL-10 (hIL-10) promoter region that was responsible for the late IL-10 expression in IECs. These results indicate that TLR4-dependent host-commensal interactions maintain the IL-10 production from IECs through 15d-PGJ2 synthesis in subepithelial M Φ s and consequent activation of PPAR γ in IECs. This may be an intrinsic immunoregulatory program that maintains mucosal homeostasis in dynamic fluctuations of epithelial permeability and in the presence of dense luminal bacteria.

Materials and Methods

Isolation of Human IECs and Lamina Propria M Φ s

Human colon specimens were obtained from surgical resection of normal margins of colon cancer in otherwise healthy individuals with the approval of the Institutional Review Board at the Cedars-Sinai Medical Center. The mucosal layer was dissected from the specimens and vigorously shaken in Ca²⁺-Mg²⁺-free HBSS containing 2 mM EDTA for 20 min at 37°C. Epithelial cells were purified from the supernatant with a 30% Percoll gradient centrifugation. Epithelial cells were further enriched by negatively depleting intraepithelial lymphocytes with magnetic beads using anti-CD45-PE and a lineage depletion kit (Miltenyi Biotec). Lamina propria mononuclear cells were isolated as previously described [24]. Lamina propria M Φ s were further purified from lamina propria mononuclear cells by magnetic sorting with anti-human CD64-PE and anti-PE microbeads (Miltenyi Biotec). Purified lamina propria

MΦs were incubated in Dulbecco's modified Eagle's medium containing 10% (vol/vol) FBS and 1% (vol/vol) penicillin-streptomycin in a 96-well plate at the concentration of 5×10^4 cells/well for 48 h in the presence or absence of lipopolysaccharide (LPS; 500 ng/ml). Supernatants were measured for 15d-PGJ2.

RT-PCR

Total RNA was extracted from purified human IECs with RNA-Bee (Tel-Test), and cDNA was amplified using a QuantiTect reverse transcription kit (Qiagen) according to the manufacturer's instructions. For PCR amplifications, we used a Phusion high-fidelity PCR mix (New England Biolabs). Primers for IL-10 were designed from the sequence from GenBank (NM 000572): sense 5'-CAGCTCAGCACTGCTCTGTTG-3' and antisense 5'-CTCCAGCAAGGACTCCTTTAAC-3'. The size of the PCR product was 211 bp. The amplification was done by 30 s denaturation at 98°C and 8 s annealing at 60.5°C for 35 cycles. Primers and amplification protocols for CD45 and β -actin have been described previously [25, 26]: CD45 sense 5'-CTGACATCATCACCTAGCAG-3' and antisense 5'-TGCTGTAGTCAATCCAGTGG-3' (product size 257 bp) and β -actin sense 5'-CATCCTCACCTGAAGTACC-3' and antisense 5'-GCTCATTGTAGAAGGTGTGG-3' (product size 92 bp). Other primers used were human TLR4 sense 5'-GCATTAACTCACTCTCCAGTC-3' and antisense 5'-CATCCTGGCTTGAGTAGATAAC-3' (product size 511 bp) and human PPAR γ sense 5'-TCTCTCCGTAATGGAAGACC-3' and antisense 5'-GCATTATGAGACATCCCCAC-3' (product size 482 bp). PCR products were visualized on a 2% agarose gel under ultraviolet light.

Cell Lines and Reagents

Human IEC lines SW480-APC and Caco-2 were kindly provided by Dr. Maree C. Faux (Ludwig Institute for Cancer Research, Australia) and Dr. Pedro Salas (University of Miami), and maintained on 12-mm Transwells (Sigma) with 0.4- μ m pore carbonate membrane insert, as previously described [27, 28]. LPS (500 ng/ml, phenol-water-extracted *Escherichia coli* K235; Sigma) was added to the bottom wells for the indicated periods. Hydrocortisone was removed during LPS stimulation in SW480-APC cells. Inhibitors for PPAR γ (SR202; R&D Systems), MAP kinases (SB203580, SP600125 and PD98059; Sigma), PI3 kinase (LY294002; Millipore) and Cox-2 (NS-398; R&D Systems) were added 90 min prior to LPS stimulation.

Isolation of Peritoneal MΦs and Co-Culture with IEC

In order to distinguish epithelial versus MΦ IL-10 production, we utilized an in vitro co-culture model of human IECs and mouse MΦs. Wild-type (WT) and TLR4 $^{-/-}$ mice on C57Bl6 background were maintained in the animal care facility at the Division of Veterinary Resources (University of Miami). All experiments described in this study have been approved by the University of Miami Institutional Animal Care and Use Committee. MΦs were isolated from peritoneal lavage of WT and TLR4 $^{-/-}$ mice using sterile PBS and seeded onto 12-well plates at a density of 2.5×10^5 cells/well with Dulbecco's modified Eagle's medium containing 10% FBS and 1% penicillin-streptomycin (Invitrogen). In the meantime, human IEC lines SW480-APC or Caco-2 cells were grown on the Transwell inserts to form a monolayer. Monolayered cells were co-cultured with or without peritoneal MΦs in the Transwell system.

Real-Time PCR

Total RNA was isolated using RNA Bee according to the manufacturer's instructions. Single-strand cDNA was synthesized from 1 μ g RNA using a QuantiTect reverse transcription kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time PCR was performed with LightCycler 480 using SYBR Premix Ex Taq (Thermo). The following primers were used: for human PPAR γ , sense primer 5'-ATCTTTCAGGGCTGCCAGT-3' and antisense primer 5'-AGTGTCTCATACGGTTTTTCGT-3'; for IL-10, sense primer 5'-GCCTAACATGCTTCGAGATC-3' and antisense primer 5'-AGTGTCTCATACGGTTTTTCGT-3', and for β -actin, sense primer 5'-CAT CCT CAC CCT GAA GTA CC-3' and antisense primer 5'-GCT CAT TGT AGA AGG TGT GG-3'. Relative expression levels were calculated with the comparative $2^{-\Delta\Delta C_t}$ method using β -actin as the endogenous control.

RNA Interference

Small interfering RNA (siRNA; 50) oligonucleotides against PPAR γ (sc-29455; Santa Cruz Biotechnology) were transfected into Caco-2 cells in a 12-well plate (1.5×10^5 cells/well) using the Lipofectamine RNAiMax transfection reagent (Invitrogen). Control cells were transfected with AllStars negative control siRNA (Qiagen). The knockdown efficiency of PPAR γ siRNA was confirmed by Western blotting.

Western Blot Analysis

Whole cell lysates were prepared from SW480-APC and Caco-2 cells using RIPA lysis buffer (Pierce Biotechnology) with a proteinase inhibitor cocktail (Calbiochem). Twenty five micrograms of the lysates were subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad). The membrane was blocked with 5% skim milk and was immunoblotted with the primary antibodies overnight at 4°C. Primary antibodies used are rabbit anti-murine Cox-2 (Cayman), rabbit anti-human PPAR γ (H-100; Santa Cruz Biotechnology) and rabbit anti- β -actin (Cell Signaling). HRP-conjugated donkey anti-rabbit IgG (Promega) was used as secondary antibody. The membrane was exposed on an X-ray film using an enhanced chemiluminescent substrate SuperSignal West Dura kit (Pierce Biotechnology).

Immunoprecipitation

SW480-APC cell lysate (400 μ g) was incubated with protein A/G plus agarose to exclude nonspecifically binding proteins (Santa Cruz Biotechnology). The liquid component was incubated with anti-PPAR γ antibody (H-100) or control normal rabbit IgG overnight at 4°C. Samples were then incubated with protein A/G plus agarose for 2 h and washed with ice-cold PBST (PBS + 0.05% Tween 20) 4 times followed by washing with ice-cold PBS. Precipitates were boiled in Laemmli sample buffer (Bio-Rad) and subjected to Western blot analysis using HRP-conjugated anti-ubiquitin (Ub) monoclonal antibody (Santa Cruz Biotechnology).

hIL-10 Promoter Cloning

Genomic DNA of SW480-APC cells was purified using an EZNA tissue DNA kit (Omega Bio-Tek) and was used as a template. Specific primers bearing restriction enzyme sites (–2,000 sense 5'-CCGCTAGCCGAGGGGGAAGGAGA-3', –1,000 sense 5'-CCGCTAGCCTCCAGCCACAGAAG-3', –500 sense 5'-CCGCTAGCTTCAAGGAGAAGT-3', –400 sense 5'-CCGCTAGCGAACACGCGAATGAGA-3' and +110 antisense 5'-GGCTC

GAGAGGTTGCCTGGGA-3') were used to generate a series of hIL-10 promoter fragments. The PCR fragments were inserted into pGL3 basic vector (Promega) via *XhoI* and *NheI* sites and ligation mixtures were delivered to JM109 as per manufacturer's instructions (Promega). Positive clones were selected and verified by DNA sequencing.

Transient Gene Expression and Reporter Assays

SW480-APC cells were plated in 12-well plates at a density of 1.5×10^5 cells/well. Reporter genes of PPAR response elements (PPRE; X3-TK-luc) that contain three copies of PPRE (Addgene), IL-10 promoter-luciferase constructs and pGL3 basic empty vector were individually co-transfected with pRL-TK (Promega) using Lipofectamine transfection reagent (Invitrogen) as per manufacturer's instructions. After overnight transfection, cells were trypsinized and transferred into 12-well Transwell inserts and grown in monolayer for 48 h. After LPS stimulation, cells were lysed with CCLR lysis buffer (Promega) and luciferase activity was measured with a Dual-Luciferase Reporter Assay (Promega). Luciferase measurements were normalized to the relative light units from Renilla luciferase from pRL-TK. Data are reported as percent increase over control samples (no LPS or pGL3 basic empty vector samples).

Chromatin Immunoprecipitation

SW480-APC cells and mouse peritoneal MΦs were prepared in the Transwell system as described above. After 48 h of LPS stimulation in the bottom wells, SW480-APC cells were harvested, and DNA and protein were cross-linked in situ with 0.5% (v/v) formaldehyde at 37°C for 5 min. Soluble chromatin was prepared using a ChIP-IT high-sensitivity kit (Active Motif). The lysates were precipitated with anti-PPARγ (H-100) or control normal rabbit IgG and protein G/A-agarose beads after sonication. DNA was purified from the precipitates and eluted DNA was amplified with sense 5'-CCGCTAGCTTAGCAAGGAGAAGT-3' and antisense 5'-GGCTCGAGAGGTTGCCTGGGA-3' primers that flanked the PPRE site of the hIL-10 promoter. PCR products were analyzed in 2.5% agarose gel.

Immunofluorescence Analysis

SW480-APC cells and mouse peritoneal MΦs were prepared in the Transwell system and stimulated with LPS for the indicated periods. SW480-APC cells on the Transwell membrane were washed with PBS and fixed in 2% paraformaldehyde overnight at 4°C. Cells were then permeabilized with 0.2% Triton X-100 (Sigma). After blocking with 10% normal goat serum for 1 h, cells were incubated with anti-PPARγ antibody (H-100) diluted in PBS containing 1% BSA (1:200) overnight at 4°C. After washing with PBS, cells were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen) diluted in PBS containing 1% BSA (1:400) for 90 min at room temperature. Nuclei were stained with DAPI (4',6-diamidino-2-phenylindole, dihydrochloride; Sigma). After washing with PBS, cells in the Transwell membrane were removed and placed on glass slides. Slides were then mounted with ProLong gold antifade reagent (Invitrogen) and analyzed with an EVOS fluorescent microscope (Advanced Microscopy Group).

Measurement of 15d-PGJ2 and IL-10 Protein

Production of 15d-PGJ2 was determined in the cell culture supernatants using EIA kit (Enzo Life Sciences) according to the manufacturer's instructions. Protein levels of hIL-10 and mouse

Fig. 1. Human primary IECs express IL-10. **a** Morphological observation of isolated IECs. Representative picture of IECs observed under the phase contrast light microscope is shown (original magnification $\times 40$, bar = 5 μ m). **b** RT-PCR analysis of mRNA expression of human IL-10, TLR4 and PPARγ in isolated IECs. Three independent IEC samples are shown (lanes 2–4). Lamina propria mononuclear cells (LPMCs, lane 1) were used as a control to prove no contamination with hematopoietic cells showing CD45 mRNA expression (middle panel).

IL-10 were determined in culture media using a DuoSet ELISA Development System (R&D Systems) following the manufacturer's instructions.

Statistical Analysis

Student's *t* test for the comparison of two independent samples and one-way ANOVA for more than two independent samples were performed using GraphPad Prism (version 5.0b); a value of $p < 0.05$ was considered statistically significant.

Results

Human Primary Colonic Epithelial Cells Express IL-10 mRNA

Previous reports have shown the expression of IL-10 in human colonic IECs by immunohistochemistry and in situ hybridization [9, 11]. In our study, we first confirmed mRNA expression of IL-10, TLR4 and PPARγ in isolated primary human IECs in the colon. Colonic IECs were purified from surgical operation samples. We confirmed the homogenous morphology of cells that represented the columnar cell phenotype (fig. 1a). Consequent analysis of human IL-10, TLR4 and PPARγ mRNA in those samples demonstrated substantial expression of these genes, while CD45 mRNA expression was negative confirming no immune cell contamination (fig. 1b). These results substantiate that normal human IECs express IL-10.

Fig. 2. Presence of MΦs amplifies IL-10 production by human IECs in response to LPS. **a** Real-time PCR results of IL-10 mRNA expression in SW480-APC cells grown in conventional wells. Cells were stimulated with LPS for the indicated periods. The relative expression of hIL-10 to β -actin is shown. Data from three independent experiments of triplicate samples with SEM (* $p < 0.05$). **b** Real-time PCR results of IL-10 mRNA expression in polarized SW480-APC cells (IEC) in the Transwell system. Cells were basolaterally stimulated with LPS for the indicated periods. One series of polarized SW480-APC cells (inserts) were co-cultured with mouse peritoneal MΦs (bottom wells). Dotted lines show results from LPS-non-treated controls. The relative expression of hIL-10 to β -actin is shown. Data from three independent experiments of triplicate samples with SEM (* $p < 0.05$). **c** IL-10 production from polarized SW480-APC cells with or without co-culturing with mouse peritoneal MΦs in the Transwell system. Cells were basolaterally stimulated with LPS as indicated. hIL-10 concentration in the bottom wells was measured by specific ELISA. Data from three

independent experiments of duplicate samples with SEM (* $p < 0.05$). **d** Real-time PCR results of IL-10 mRNA expression in polarized SW480-APC cells during co-culturing with human monocyte/MΦ line U937 cells in the Transwell system. Cells were basolaterally stimulated with LPS for the indicated periods. The relative expression of hIL-10 to β -actin is shown. Data from three independent experiments of triplicate samples with SEM (* $p < 0.05$). **e** IL-10 production from mouse peritoneal MΦs during co-culture with SW480-APC cells in the Transwell system. Cells were basolaterally stimulated with LPS as indicated. Mouse IL-10 (mIL-10) concentration in the bottom wells was measured by specific ELISA. Data from two independent experiments of duplicate samples with SEM (* $p < 0.05$). **f** ELISA detection of hIL-10 without cross-reaction to mouse IL-10. Black bars show concentration of IL-10 (human: wells A, B, C, mouse: wells F, G, H) added and white bars show optical density (OD) measured by a spectrophotometer. Wells D and E received no IL-10 (background).

MΦs Enhance IL-10 Production from Human IECs during LPS Stimulation

The physiological relevance of the expression of IL-10 in IECs has been shown in ex vivo human intestinal mucosal culture [9]. In this study, we used SW480-APC cells as an IEC line because it polarizes on the Transwell and expresses functional TLR4 [27]. TLR4 stimulation of this cell line in the conventional wells resulted in a significant increase in IL-10 mRNA expression in 24 h (fig. 2a). In a

time course experiment, polarized SW480-APC cells showed a significant increase in IL-10 expression, which peaked 24 h after basolateral stimulation with LPS. When we co-cultured polarized SW480-APC cells with peritoneal MΦs in Transwells, IL-10 expression by IECs had another peak at 72 h of basolateral LPS stimulation, which was two times greater than the original peak at 24 h (fig. 2b). This enhancement in IL-10 expression by MΦ co-culture was also confirmed at the protein level (fig. 2c).

The late induction of IL-10 mRNA by IECs was also observed when we co-cultured polarized SW480-APC cells with the human monocyte/MΦ cell line U937 (fig. 2d). On the other hand, the production of IL-10 from MΦs in response to LPS stimulation did not change by co-culturing with IECs (fig. 2e). Our experimental system co-culturing polarized human IECs with mouse peritoneal MΦs is advantageous to distinguish IEC IL-10 production from MΦ IL-10 production (fig. 2f). Therefore, these results suggest that the expression of IL-10 from human IECs is induced by humoral substances from MΦs during LPS exposure.

MΦ TLR4 Signaling Induces Anti-Inflammatory Prostaglandin 15d-PGJ2 during Co-Culturing with IECs

Next we tried to identify the humoral substance produced by MΦs in response to LPS. We have shown that one of the major intestinal phenotypes of TLR4-deficient mice is impaired expression of Cox-2 in subepithelial MΦs [14, 29]. The decreased Cox-2 induction results in defective mucosal synthesis of anti-inflammatory 15d-PGJ2 during mucosal injury [14]. Since 15d-PGJ2 is known to support the expression of anti-inflammatory cytokines by inhibiting intracellular proinflammatory pathways, we tested whether 15d-PGJ2 was produced by MΦs in response to LPS. Stimulation of MΦs with LPS during co-culturing with IECs resulted in a significant induction of 15d-PGJ2 in the bottom wells (i.e. by MΦs) at the peak of 48 h (fig. 3a). We confirmed that MΦs were the major source of 15d-PGJ2 in our co-culture system as we observed a consistent concentration of 15d-PGJ2 regardless of the presence or absence of IECs (fig. 3b). Furthermore, 15d-PGJ2 production was not observed when we used TLR4-deficient MΦs (fig. 3c). The inhibition of Cox-2 (by NS398) or MAP kinases p38 (by SB203580) and ERK (by PD98059) individually suppressed 15d-PGJ2 production by MΦs, while 15d-PGJ2 production was independent of JNK (SP600125) and PI3 kinases (LY294002; fig. 3c). Corresponding to the 15d-PGJ2 production, we confirmed Cox-2 induction in MΦs within 24 h of LPS stimulation (fig. 3d). Furthermore, we observed substantial induction of 15d-PGJ2 in freshly isolated primary human colonic MΦs after stimulation with LPS (fig. 3e).

In contrast to 15d-PGJ2, mRNA expression of the proinflammatory cytokine TNF- α was rapidly induced by MΦs within 4 h of LPS stimulation and TNF- α induction was down-regulated after 24 h (fig. 3f). This induction of MΦ TNF- α was significantly suppressed when co-cultured with IECs. Induction of TNF- α mRNA expression

from IECs was not observed after LPS stimulation (data not shown). These results indicate that MΦs induce anti-inflammatory 15d-PGJ2 in response to LPS through TLR4-mediated Cox-2 induction which requires p38 and ERK MAP kinase activation. By contrast, TLR4-mediated expression of proinflammatory TNF- α in MΦs is rapid and transient, which may be down-regulated by co-culturing with IECs.

LPS Stimulation Induces PPAR γ Activation in Human IECs during Co-Culturing with MΦs

Prostaglandins are functionally conserved among species and 15d-PGJ2 is a natural ligand of PPAR γ [30, 31]. We tested whether the 15d-PGJ2 produced by mouse MΦs activates PPAR γ in human IECs. First, we confirmed the protein expression of PPAR γ in our IEC line SW480-APC. Western blot analysis demonstrated an increase in PPAR γ protein expression in IECs after stimulation with LPS (fig. 4a). There was no significant difference in the expression of PPAR γ mRNA after LPS stimulation (Figure 4b). In contrast, ubiquitination of PPAR γ protein was reduced by 24 h after LPS stimulation (fig. 4c). Therefore, the increased expression of PPAR γ protein was not likely to be corresponding with PPAR γ mRNA induction but rather associated with reduced degradation of PPAR γ protein.

Next, we examined PPAR γ activation in IECs during co-culturing with MΦs. SW480-APC cells were transfected with a PPARE-luciferase reporter construct then co-cultured with mouse MΦs in the presence of LPS. A significant increase in PPAR γ transcriptional activity was observed 48 h after LPS stimulation (fig. 5a). Such an increase in PPAR γ activation was not observed without co-culturing with MΦs (fig. 5b). Moreover, MΦ TLR4 signaling was responsible for the induction of PPAR γ activation in IECs in response to LPS because LPS stimulation failed to activate PPAR γ in IECs when we used TLR4-deficient MΦs (fig. 5c). Consistently, immunofluorescent detection of cellular localization of PPAR γ demonstrated nuclear accumulation of PPAR γ 48 h after LPS stimulation (fig. 5d).

These results suggest that LPS stimulation of IEC-MΦ co-culture leads to accumulation and activation of PPAR γ in IECs by inhibiting proteasomal degradation of PPAR γ in IECs and 15d-PGJ2 synthesis by MΦs, respectively.

IL-10 Promoter Isolated from Human IECs Has PPARE Motifs

We examined whether the activated PPAR γ directly induces IL-10 expression in IECs by cloning the hIL-10

Fig. 3. LPS stimulates MΦ 15d-PGJ2 synthesis through TLR4-dependent activation of p38, ERK MAP kinases and Cox-2 induction during co-culturing with IECs. **a** 15d-PGJ2 synthesis in IEC-MΦ co-culture. Polarized SW480-APC cells were co-cultured with mouse peritoneal MΦs in the Transwell system. LPS was added to the bottom wells. Supernatant from the bottom wells was measured for 15d-PGJ2 by EIA at the indicated periods. Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **b** 15d-PGJ2 synthesis by cell types. Mouse peritoneal MΦs were co-cultured with or without polarized SW480-APC cells (IECs) in the Transwell system. LPS was added to the wells of MΦs. Polarized IECs without MΦs were basolaterally stimulated with LPS as indicated. Supernatant from the bottom wells was measured for 15d-PGJ2 at the indicated periods. Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **c** 15d-PGJ2 synthesis during inhibition of Cox-2 or PI3 and MAP kinases. Polarized SW480-APC cells were co-cultured with mouse peritoneal MΦs in the Transwell system. To block TLR4 signaling, some wells were prepared with MΦs from TLR4-/- mice. Inhibitors for p38 (SB203580; 20 μ M), JNK (SP600125; 20 μ M), ERK (PD98059; 20 μ M), PI3K (LY294002; 20 μ M) and Cox-2

(NS398; 5 μ M) were added to the bottom wells. Ninety minutes later, LPS was added to the bottom wells. Supernatant from the bottom wells was measured for 15d-PGJ2 after 48 h. Data from two independent experiments of triplicate samples with SEM (* $p < 0.05$). **d** Western blot analysis of Cox-2 in MΦs. Mouse peritoneal MΦs were co-cultured with SW480-APC cells in the Transwell system. Protein samples were harvested from MΦs before and after 24 h of LPS stimulation in the bottom wells. An equal amount of protein was loaded per lane. The membrane was sequentially probed for Cox-2 and β -actin. Representative results from three repeated experiments are shown. **e** 15d-PGJ2 synthesis in human colonic MΦs. Freshly isolated lamina propria MΦs (CD64 $^{+}$ cells) were stimulated with LPS for 48 h. 15d-PGJ2 concentrations in supernatants were measured by EIA. Data of triplicate samples from 2 human subjects with SEM (* $p < 0.05$). **f** Real-time PCR results of TNF- α mRNA expression (mTNF- α) in mouse peritoneal MΦs with or without co-culturing with IECs in the Transwells. The relative expression of mouse TNF- α to β -actin after LPS stimulation is shown. Data from three independent experiments of triplicated samples with SEM (* $p < 0.05$).

Fig. 4. Epithelial TLR4 signaling inhibits degradation of PPAR γ protein. **a** Western blot analysis of PPAR γ in SW480-APC cells. SW480-APC cells were stimulated with LPS in conventional wells for the indicated periods. An equal amount of protein was loaded per lane. The membrane was sequentially probed for PPAR γ and β -actin. Representative results from three repeated experiments are shown. **b** Real-time PCR results in PPAR γ mRNA expression in SW480-APC cells grown in conventional wells. Cells were stimulated with LPS for the indicated period. The relative expression of human PPAR γ to β -actin is shown. Data from three independent experiments of triplicate samples with SEM. n.s. = Non-significant. **c** Detection of ubiquitinated PPAR γ by immunoprecipitation. Polarized SW480-APC cells were co-cultured with mouse peritoneal M Φ s and basolaterally stimulated with LPS for the indicated periods. 400 μ g of protein from SW480-APC cell lysates were immunoprecipitated with anti-PPAR γ or control IgG and immunoblotted with HRP-conjugated anti-Ub monoclonal antibody (P4D1), followed by anti-PPAR γ (bottom). Representative results from three repeated experiments are shown. Graph shows quantitative Ub band (each molecular weight level) intensity normalized with PPAR γ band measured with ImageJ.

promoter in SW480-APC genomic DNA. PPAR γ binds as heterodimer with members of the retinoid X receptor family to PPREs that are composed of a direct repeat of two half sites of the consensus sequences [32]. Based on previous reports, we found two possible PPRE motifs in the hIL-10 promoter (406–394 and 521–509 bp upstream from the ATG start codon; fig. 6a). To confirm the PPAR γ binding sites within the hIL-10 promoter, we generated four luciferase reporter constructs serially truncated at

the 5' end of the IL-10 promoter. These reporter constructs were transfected to SW480-APC cells and examined for IL-10 promoter activities in response to LPS stimulation during co-culturing with M Φ s. All the constructs that carried a PPRE motif in the 406- to 394-bp upstream region showed significant IL-10 promoter activity (fig. 6b). The construct truncated at the region from –521 to –509 (–500) still showed IL-10 promoter activity in response to LPS but the truncation deleted at the region

Fig. 5. M Φ TLR4 signaling induces activation of PPAR γ in IECs during IEC-M Φ co-culture. **a** PPAR γ activation in SW480-APC cells during co-culturing with M Φ s. SW480-APC cells were transfected with a PPRE-luciferase reporter construct and co-cultured with M Φ s in the Transwell system. LPS was added to the bottom wells for the indicated periods. PPAR γ activation was expressed as percent increase of PPRE-luciferase activity over control (no LPS). Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **b** PPAR γ activation in SW480-APC cells without M Φ co-culture. SW480-APC cells were transfected with a PPRE-luciferase reporter construct and plated in the Transwell insert. Cells were basolaterally stimulated with LPS as indicated. PPAR γ activation was expressed as percent increase of PPRE-luciferase activity over control (no LPS). Data from three independent experiments of duplicate samples with SEM. n.s. = Nonsig-

nificant. **c** Involvement of M Φ TLR4 signaling in PPAR γ activation of SW480-APC cells. SW480-APC cells were transfected with a PPRE-luciferase reporter construct and co-cultured with mouse peritoneal M Φ s isolated from TLR4 $^{-/-}$ mice or WT littermates in the Transwell system. LPS was added to the bottom wells. PPAR γ activation was measured after 48 h as percent increase in PPRE-luciferase activity over control (no LPS). Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **d** Immunofluorescent detection of cellular localization of PPAR γ . Polarized SW480-APC cells were co-cultured with mouse peritoneal M Φ s in the Transwell system and basolaterally stimulated with LPS for the indicated periods. PPAR γ was labeled with Alexa Fluor 488 (green in the online version). Nuclei were stained with DAPI (blue in the online version). Original magnification $\times 40$. Bars: 50 μ m.

from -406 to -394 abolished the LPS-mediated IL-10 promoter activity (fig. 6b). Therefore, PPRE in the region from -406 to -394 of the IL-10 promoter is responsible for PPAR γ -dependent IL-10 promoter activity. Direct stimulation of SW480-APC cells carrying full-length IL-

10 promoter with 15d-PGJ2 (5 ng/ml for 48 h) in the absence of LPS did not show increased luciferase activity (data not shown), suggesting the requirement of TLR4-mediated PPAR γ expression for transcriptional activity of IL-10 in IECs.

Fig. 6. Detection of functional PPRE motif in IL-10 promoter in human IEC. **a** hIL-10 promoter sequence in genomic DNA isolated from SW480-APC cells. PPRE motifs are underlined. **b** Detection of a functional PPRE element in hIL-10 promoter. SW480-APC cells were transfected with truncated hIL-10 promoter fragments of various lengths and co-cultured with mouse peritoneal MΦs in the Transwell system. LPS was added to the bottom wells and IL-10 promoter activity was measured after 72 h as percent increase of luciferase activity over control (empty vector). Data

from three independent experiments of duplicate samples with SEM (** $p < 0.01$, *** $p < 0.001$). n.s. = Nonsignificant. **c** The binding of PPARγ to hIL-10 promoter. Polarized SW480-APC cells were co-cultured with mouse peritoneal MΦs in the Transwell system and basolaterally stimulated with LPS for 72 h as indicated. Nuclear protein was isolated from LPS-stimulated and non-stimulated SW480-APC cells and subjected to ChIP assay. Normal rabbit IgG was used for immunoprecipitation of control samples. Representative results from three repeated experiments are shown.

To further confirm the direct binding of PPARγ to the region from -406 to -394 of the IL-10 promoter, we performed a ChIP assay. Nuclear protein was immunoprecipitated with PPARγ and PCR amplified using a primer set that was used to clone the -500-bp hIL-10 promoter construct. We detected this region of hIL-10 promoter in the DNA fragment that bound with PPARγ in LPS-stimulated SW480-APC cells (fig. 6c). These results indicate that activated PPARγ in SW480-APC cells directly binds to the PPRE motif in the IL-10 promoter, which is induced by LPS stimulation during co-culturing of SW480-APC cells with MΦs.

MΦ TLR4-Mediated Late Induction of IL-10 in IECs Is PPARγ Dependent

Next, we addressed whether the observed IL-10 promoter activity is PPARγ specific and the PPARγ binding to IL-10 promoter results in IL-10 expression in SW480-APC cells during co-culturing with MΦs. Blocking PPARγ by the PPARγ antagonist SR202 abolished LPS-mediated IL-10 promoter activity and the late induction of IL-10 mRNA in our SW480-APC MΦ co-culture system (fig. 7a, b). This effect of SR202 on IL-10 expression was not observed without MΦs, confirming that the activation of PPARγ in SW480-APC cells requires a MΦ re-

Fig. 7. The late induction of IL-10 in IEC-MΦ co-culture is epithelial PPAR γ dependent. **a** IL-10 promoter activity. SW480-APC cells were transfected with -2,000-bp hIL-10 promoter and co-cultured with mouse peritoneal MΦs in the Transwell system. LPS was added to the bottom wells and IL-10 promoter activity was measured after 72 h as percent increase in luciferase activity over control (no LPS). The PPAR γ antagonist SR202 (500 μ M) was added to insert wells 90 min prior to LPS stimulation. Data from two independent experiments of duplicate samples with SEM (* $p < 0.05$). **b** Real-time PCR results of IL-10 mRNA expression in polarized SW480-APC cells in the presence or absence of PPAR γ antagonist. Polarized SW480-APC cells with or without mouse peritoneal MΦs were basolaterally stimulated with LPS for 72 h. PPAR γ antagonist SR202 (500 μ M) was added to insert wells 90 min prior to LPS stimulation. The relative expression of hIL-10 to β -actin is shown. Data from three independent experiments of triplicate samples with SEM (* $p < 0.05$). n.s. =

Nonsignificant. **c** The effect of SR202 on IL-10 production from polarized SW480-APC cell co-culturing with mouse peritoneal MΦs in the Transwell system. Cells were basolaterally stimulated with LPS for 72 h as indicated. PPAR γ antagonist SR202 (500 μ M) was added to insert wells 90 min prior to LPS stimulation. hIL-10 protein concentration in the bottom wells was measured by specific ELISA. Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **d** The effect of SR202 on PPAR γ activation in SW480-APC cells during co-culturing with MΦs. SW480-APC cells were transfected with a PPARE-luciferase reporter construct and co-cultured with mouse peritoneal MΦs in the Transwell system. LPS was added to the bottom wells for 48 h as indicated. The PPAR γ antagonist SR202 (500 μ M) was added to insert wells 90 min prior to LPS stimulation. PPAR γ activation was expressed as percent increase in PPARE-luciferase activity over control (no LPS). Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$).

sponse to LPS (fig. 7b). Corresponding IL-10 protein production by SW480-APC cells was also abrogated by SR202 (fig. 7c). Furthermore, the IL-10 expression pattern was comparable to the PPAR γ transcriptional activity determined by PPARE-luciferase assay (fig. 7d). These results indicate that the late IL-10 expression by SW480-APC cells depends on PPAR γ activation in SW480-APC cells, which requires MΦ TLR4 signaling.

Epithelial-MΦ Crosstalk Induces IL-10 Expression in IECs via MΦ Cox-2 Induction and Epithelial PPAR γ Activation

In order to map the cellular pathway in epithelial-MΦ crosstalk that evokes epithelial IL-10 expression in response to LPS, we utilized another human IEC line (Caco-2) that is known to be hyporesponsive to LPS and constitutively expresses PPAR γ (fig. 8a). Using Caco-2 cells allowed us to investigate IEC-MΦ crosstalk without the effect of IECs on MΦs that were induced by LPS stimulation. Caco-2 cells were polarized in the Transwell inserts and co-cultured with peritoneal MΦs that were seeded in the bottom wells. Consistent with SW480-APC cells,

Caco-2 cells induced IL-10 expression 72 h after adding LPS in the bottom wells, but early induction of IL-10 in 24-hour LPS stimulation was not observed (fig. 8b). Corresponding IL-10 protein expression was confirmed by ELISA, which was not observed without MΦs (fig. 8c). In this system, knocking down PPAR γ completely abolished LPS-mediated IL-10 transcriptional activity determined by IL-10 promoter luciferase assay (fig. 8d). 15d-PGJ2 stimulation induced IL-10 promoter activity in Caco-2 cells, which was not seen in SW480-APC cells, confirming the requirement of PPAR γ in 15d-PGJ2-mediated IL-10 expression in human IECs. The IL-10 transcriptional activity reflected IL-10 protein expression in Caco-2 cells (fig. 8e). Although PPAR γ is constitutively expressed in Caco-2 cells, its transcriptional activity is induced by TLR4-mediated Cox-2 induction in MΦs as the presence of Cox-2 inhibitor (NS398) completely abolished LPS-induced PPARE activity in Caco-2 cells during co-culturing with MΦs (fig. 8f). These results suggest that IL-10 expression from human IECs is PPAR γ dependent and inducible by activation of adjacent MΦs through TLR4-mediated Cox-2 induction.

Fig. 8. The role of epithelial PPAR γ activation in IL-10 expression by IECs that are induced by M Φ TLR4 signaling. **a** Western blot analysis of PPAR γ in Caco-2 cells. Caco-2 cells were stimulated with LPS in conventional wells for the indicated periods. An equal amount of protein was loaded per lane. The membrane was sequentially probed for PPAR γ and β -actin. Representative results from three repeated experiments are shown. Graph shows ratios of PPAR γ band intensity normalized with β -actin band measured with ImageJ. **b** Real-time PCR results of IL-10 mRNA expression in polarized Caco-2 cells. Polarized Caco-2 cells were co-cultured with mouse peritoneal M Φ s in the Transwell system and basolaterally stimulated with LPS for the indicated periods. The relative expression of hIL-10 to β -actin is shown. Data from three independent experiments of triplicate samples with SEM (* $p < 0.05$). **c** IL-10 production from polarized Caco-2 cells with or without co-culturing with mouse peritoneal M Φ s in the Transwell system. Cells were basolaterally stimulated with LPS for the indicated periods. hIL-10 protein concentration in the bottom wells was measured by specific ELISA. Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **d** The role of PPAR γ in the transcriptional activity of IL-10 in human IECs. Caco-2 cells were transfected with hIL-10 promoter construct together with siRNA against PPAR γ and co-cultured with mouse peritoneal M Φ s in the Transwell system. Control cells received IL-10 promoter construct

and nonspecific siRNA. LPS was added to the bottom wells and IL-10 promoter activity was measured after 72 h as percent increase in luciferase activity over control (no LPS, control siRNA). The extent of PPAR γ knockdown was assessed by Western blot. 15d-PGJ2 (5 ng/ml) was used as a positive control. Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). n.s. = Nonsignificant. **e** IL-10 production from polarized Caco-2 cells knocking down PPAR γ . Caco-2 cells were transfected with siRNA against PPAR γ and co-cultured with mouse peritoneal M Φ s in the Transwell system. Control cells were transfected with nonspecific siRNA. Cells were basolaterally stimulated with LPS for 72 h as indicated. hIL-10 concentration in the bottom wells was measured by specific ELISA. Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$). **f** The effect of M Φ Cox-2 induction on PPAR γ activation in Caco-2 cells during co-culturing with M Φ s. Caco-2 cells were transfected with a PPARE-luciferase reporter construct and co-cultured with mouse peritoneal M Φ s in the Transwell system. LPS was added to the bottom wells for 48 h as indicated. Cox-2 antagonist NS398 (5 μ M) was added to the bottom wells 90 min prior to LPS stimulation. PPAR γ activation was expressed as percent increase in PPARE-luciferase activity over control (no LPS). Data from three independent experiments of duplicate samples with SEM (* $p < 0.05$).

Discussion

Intestinal mucosal immune responses to luminal commensal microorganisms are regulated by active immunomodulation in which the expression of anti-inflammatory cytokines from IECs maintains a controlled state of inflammation. While the importance of epithelial IL-10 expression in keeping epithelial integrity has been described in the intestine, the precise mechanism regulating epithelial IL-10 expression in the context of facing numerous commensal microorganisms has remained elusive. In this study, we show an important role of TLR4-mediated crosstalk between IECs and MΦs in epithelial expression of IL-10. The underlying mechanism of epithelial IL-10 production involves activation of the nuclear receptor PPAR γ in IECs. On the other hand, TLR4 signaling in MΦs induces 15d-PGJ2 synthesis via Cox-2 expression that is required for PPAR γ activation in adjacent IECs. These results outline a sequential molecular and cellular pathway by which TLR4 signaling coordinates intestinal immunomodulation in response to commensal bacteria.

In normal intestinal mucosa, MΦs are located underneath the epithelial lining [33]. It has been reported that these subepithelial MΦs crosstalk with adjacent IECs through TLR-dependent Cox-2 expression [15, 16]. While intestinal MΦs isolated from human jejunum are known to be unresponsive to LPS mainly due to lack of CD14 expression, CD14-expressing MΦs are identified in normal human colonic mucosa [34]. Their response to LPS seems to be regulated by retinaldehyde dehydrogenase activity, and abnormal responses of this MΦ population to commensal bacteria has been implicated in the pathogenesis of human IBD [34–36]. How these potentially LPS-responsive lamina propria MΦs are regulated in normal colonic mucosa is obscure, but induction of immunomodulatory 15d-PGJ2 may contribute to the regulation of inflammatory responses of LPS-activated MΦs to keep mucosal homeostasis in the normal colon. Since Cox-2 is known to induce prostaglandin synthesis, we examined whether MΦs produced 15d-PGJ2 in response to TLR4 activation. We confirmed a significant induction of Cox-2 followed by 15d-PGJ2 synthesis in LPS-stimulated MΦs that is mediated through p38 and ERK1/2 MAP kinases. We also confirmed induction of 15d-PGJ2 in primary colonic MΦs (CD64 $\pm$ cells) in response to LPS. It is likely that MΦs are the major source of 15d-PGJ2 in the intestine because Cox-2 expression is mainly observed in lamina propria MΦs [29], and the amount of 15d-PGJ2 produced is similar in the presence

and absence of IECs in our co-culture system. In addition, IECs increased intracellular PPAR γ protein in response to LPS. This corresponded to the significant reduction in ubiquitination of PPAR γ protein. The Ub-dependent proteasome pathway is known to regulate several transcription factors, including PPAR γ [37]. The Ub-dependent degradation of PPAR γ not only regulates intracellular protein levels but also directly influences PPAR γ activation as the ubiquitination occurs in the ligand-binding domain of PPAR γ [38]. Furthermore, 15d-PGJ2 has been shown to inhibit the Ub-dependent proteasome pathway in IECs [39].

Although LPS stimulation has shown to induce PPAR γ mRNA expression in Caco-2 cells, neither SW480-APC cells nor Caco-2 cells showed a significant difference in PPAR γ mRNA before and after LPS stimulation in our system [22]. This discrepancy may be due to differences in transcriptional responses of each PPAR γ mRNA isoform in that IECs express multiple PPAR γ mRNA isoforms that use different promoters [40]. Since each PPAR γ mRNA isoform has different sequences in the N-terminal region, different primer sets detect distinct isoforms of PPAR γ mRNA. In our study, we used a primer set that can detect the universal region of PPAR γ mRNA. Functionally, the existence of MΦs was required for PPAR γ activation in IECs because we could not see LPS-induced PPAR γ activation and nuclear accumulation in IECs when they were not co-cultured with MΦs. Overall, our results indicate that TLR4-mediated IEC-MΦ crosstalk triggers epithelial PPAR γ activation through Cox-2 induction and 15d-PGJ2 production from MΦs.

Animal studies have demonstrated the importance of IL-10 in the maintenance of host immune responses to intestinal commensal bacteria [6, 7]. A recent report has also shown that conditional deletion of epithelial-specific IL-10 in mice results in increased susceptibility to oxazolone-induced colitis [41]. However, the role of IECs as a source of IL-10 differs between mice and humans because mouse IECs do not constitutively express IL-10 without stimulation [10]. In human IECs, we confirmed the expression of IL-10 mRNA in isolated normal colonic epithelial cells from surgically resected specimens. It has been shown that the expression of IL-10 maintains epithelial integrity by suppressing the expression of detrimental proinflammatory cytokines and apoptotic cell death [9, 42]. These findings imply unique regulation of IL-10 expression in human IECs. In general, gene expression is tightly regulated by binding of a group of transcription factors to specific promoter regions of the gene, which differs among cell types [43]. Several transcription

factors, such as Sp1, Sp3, NF- κ B, STAT3 and C/EBP, are known to bind to the hIL-10 promoter in monocytes and M Φ s [44–46]. Contribution of PPAR γ in the transcriptional regulation of human M Φ IL-10 promoter activity has also been shown [47]. However, little is known about transcriptional regulation of IL-10 in human IECs. We found in this study that LPS stimulation induces two phases of IL-10 expression by IECs during co-culturing with M Φ s. The first phase could be seen without M Φ s and was prior to the nuclear accumulation of PPAR γ in IECs, indicating that the first phase of IL-10 expression is independent of PPAR γ , while the second phase was PPAR γ dependent and required Cox-2 induction in M Φ s. Although it is still unclear whether these two phases of IL-10 expression have distinct roles in the maintenance of host immune responses to intestinal bacteria, PPAR γ activation via TLR4-mediated epithelial-M Φ crosstalk may be important to keep a biologically active level of IL-10 in the intestinal mucosa since the level of IL-10 induction is greater in the second rather than the first phase. Based on these findings, we propose an immunological model in which TLR4-mediated crosstalk between IECs and M Φ s maintains mucosal integrity in the human colon. Upon minor mucosal injury of the colon, innate proinflammatory responses may be initiated rapidly, while sequential induction of innate anti-inflammatory responses composed of 15d-PGJ2 and the late induction of epithelial IL-10 gives enough time to prepare a host-defense program against possible invasion of luminal microorganisms by regulating inflammatory overdrive until more precise adaptive immunity is mobilized.

Unlike other organs in our body, the gastrointestinal tract houses countless bacteria. In order to keep this unique nature of the gut, active and sustained immunoregulation is crucial to dampen intestinal inflammatory signaling in response to commensal bacteria. We demonstrated in this study that TLR4-induced IEC-M Φ crosstalk leads to the activation of PPAR γ and IL-10 production in IECs. Given the importance of IL-10 in the maintenance of our intestinal homeostasis, it is interesting that this IEC-M Φ crosstalk relies on commensal bacteria. Many disease conditions in the gastrointestinal tract have been expected to involve the host-bacterial interactions. While IECs are normally hyporesponsive to apical bacterial stimuli, epithelial leakage may occur upon a variety of physiological conditions leading to initiation of host response to microbial products, which has been implicated as a predisposing factor of irritable bowel disease and IBD [4, 5, 48, 49]. Our results outline the physiological mechanism of intestinal host-microbial interactions, which may provide cues to understand the pathogenesis of gastrointestinal diseases that involve abnormal host response to commensal bacteria.

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Disclosure Statement

The authors have no conflicting financial interests.

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