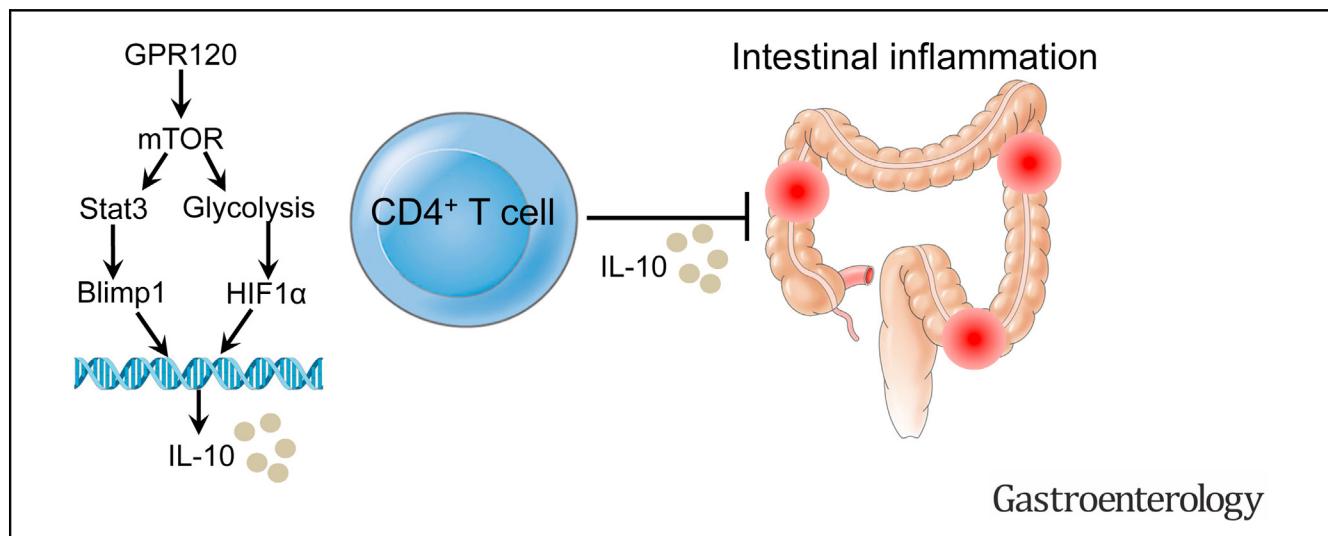




GPR120 Inhibits Colitis Through Regulation of CD4⁺ T Cell Interleukin 10 Production

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BACKGROUND & AIMS: G protein-coupled receptor (GPR) 120 has been implicated in regulating metabolic syndromes with anti-inflammatory function. However, the role of GPR120 in intestinal inflammation is unknown. Here, we investigated whether and how GPR120 regulates CD4⁺ T cell function to inhibit colitis development. **METHODS:** Dextran sodium sulfate (DSS)-induced colitis model, *Citrobacter rodentium* infection model, and CD4⁺ T cell adoptive transfer model were used to analyze the role of GPR120 in regulating colitis development. The effect of GPR120 on CD4⁺ T cell functions was analyzed by RNA sequencing, flow cytometry, and Seahorse metabolic assays. Mice were administered GPR120 agonist for investigating the potential of GPR120 agonist in preventing and treating colitis. **RESULTS:** Deficiency of GPR120 in CD4⁺ T cells resulted in more severe colitis in mice upon dextran sodium sulfate insult and enteric infection. Transfer of GPR120-deficient

CD4⁺CD45Rb^{hi} T cells induced more severe colitis in *Rag*^{-/-} mice with lower intestinal interleukin (IL) 10⁺CD4⁺ T cells. Treatment with the GPR120 agonist CpDA promoted CD4⁺ T cell production of IL10 by up-regulating Blimp1 and enhancing glycolysis, which was regulated by mTOR. GPR120 agonist-treated wild-type, but not IL10-deficient and Blimp1-deficient, T helper 1 cells induced less severe colitis. Furthermore, oral administration of GPR120 agonist protected mice from intestinal inflammation in both prevention and treatment schemes. *Gpr120* expression was positively correlated with *Il10* expression in the human colonic mucosa, including patients with inflammatory bowel diseases. **CONCLUSIONS:** Our findings show the role of GPR120 in regulating intestinal CD4⁺ T cell production of IL10 to inhibit colitis development, which identifies GPR120 as a potential therapeutic target for treating inflammatory bowel diseases.

Keywords: Effector CD4⁺ T cells; Glycolysis; Blimp1; Intestinal Homeostasis; Inflammatory Bowel Diseases.

It has been well established that CD4⁺ T cell responses to gut microbiota play crucial roles in the pathogenesis of inflammatory bowel disease (IBD). Among CD4⁺ T cells, T helper (Th) 1 and Th17 cells are central to the pathogenesis of certain types of IBD,¹ which can be inhibited by multiple mechanisms, including CD4⁺ T cell production of interleukin (IL) 10, a critical immunosuppressive cytokine for regulating intestinal homeostasis. Polymorphisms in the *Il10* locus confer risk for IBD.^{2,3} Deficiency in IL10 or IL10 receptor (IL10R) in mice and humans causes severe intestinal inflammation.^{4–6} IL10-IL10R signaling is essential in regulatory T cells (Tregs) for their suppressive functions in IBD, in CD4⁺ T effector cells for inhibiting exaggerated T cell responses in mucosal compartments, and also in innate cells for regulating mucosal homeostasis.^{7–9} However, how CD4⁺ T cell production of IL10 is regulated is still not completely understood.

Accumulating evidence indicates that dietary compositions regulate health and disease, regardless of energy intake.¹⁰ It has been shown that dietary fatty acids, the primary dietary components, affect host immune responses.¹¹ Long-chain fatty acids are the major dietary fatty acids absorbed and sensed by host cells. Omega 3 fatty acids (ω -3 FA), which belong to the group of long-chain fatty acids and are commonly consumed as fish products, dietary supplements, and pharmaceuticals, have been shown to have potent anti-inflammatory effects and several health benefits, including amelioration of atherosclerosis and increased insulin sensitivity.^{12,13} The intestinal tract directly interacts with dietary components.¹⁴ High dietary ω -3 FA has been associated with a low risk of IBD.¹⁵ However, the underlying mechanisms are still unclear. G protein-coupled receptor (GPR) 120, which was recently identified as a receptor for ω -3 FA,¹⁶ has a critical role in various physiologic homeostasis processes such as adipogenesis.^{17,18} Deficiency of GPR120 leads to obesity in both humans and mice, and *Gpr120*^{-/-} mice are more susceptible to insulin resistance and have higher expression of the genes related to inflammation,¹⁹ whereas GPR120 agonists inhibit proinflammatory cytokine production by dendritic cells (DCs) and macrophages.^{16,20} However, it is still unknown how GPR120 regulates the pathogenesis of IBD.

In this study, we show that *Gpr120*^{-/-} mice and CD4⁺ T cell-specific GPR120-knockout *Cd4*^{cre}*Gpr120*^{fl/fl} mice develop more severe colitis than wild-type (WT) mice. *Gpr120*^{-/-}CD4⁺ T cells induce more severe colitis than WT CD4⁺ T cells when transferred into *Rag*^{-/-} mice. The GPR120 agonist promotes CD4⁺ T cell production of IL10 through up-regulation of Blimp1 and enhancing glycolysis. Importantly, *Gpr120* expression is positively correlated with *Il10* expression in the human mucosa, including patients with IBD.

Materials and Methods

Mice

C57BL/6J WT mice, B6.129-Prdm1tm1Clme/J (*Prdm*^{fl/fl}) mice, B6.Cg-Tg(*Cd4*-cre)1Cwi/BfluJ (*Cd4*^{cre}) mice, and B6.SJL-Ptprc^a Pepc^b/BoyJ (CD45.1) mice were purchased from The

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

G protein-coupled receptor (GPR) 120 has been implicated in regulating metabolic syndromes with anti-inflammatory function. However, the role of GPR120 in the regulation of intestinal inflammation is unknown.

NEW FINDINGS

GPR120 induces interleukin 10 production in CD4⁺ T cells through up-regulation of Blimp1 and enhancing glycolysis to inhibit colitis. Administration of GPR120 agonist protected mice against intestinal inflammation.

LIMITATIONS

A limitation of the current study is that it was mostly performed in animal models. The effect of GPR120 in human CD4⁺ T cells and patients with inflammatory bowel disease needs to be further validated in the clinical setting.

IMPACT

GPR120 is identified as a potential therapeutic target for treating inflammatory bowel disease.

Jackson Laboratory. *Gpr120*^{-/-} mice were provided by Bristol Myers Squibb. *Gpr120*^{fl/fl} mice were generated by inserting 2 loxP sites in the *Gpr120* allele using CRISPR/Cas9 gene-editing technology in the C57BL/6J background in Viewsolid Biotech and crossed to *Cd4*^{cre} to generate *Cd4*^{cre}*Gpr120*^{fl/fl} mice. IL10^{-/-} mice were provided by Dr Cohn at the University of the Texas Medical Branch (UTMB). *Prdm*^{fl/fl} mice were crossed to *Cd4*^{cre} to generate *Cd4*^{cre}*Prdm*^{fl/fl} mice. All mice were maintained on a 12-hour light/dark cycle with the temperature of 20°C–26°C and 30%–70% humidity in the specific pathogen-free animal facility of UTMB. All the experimental mice were sex-matched and age-matched littermates and cohoused after weaning. All the animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of UTMB.

Human

All patients with Crohn's disease (CD) and ulcerative colitis (UC) were recruited at the Gastroenterology Department of the First Affiliated Hospital of Nanjing Medical University. The diagnosis of CD and UC was based on the combination of clinical signs and symptoms, endoscopic features, and histologic results. Colonic tissues were collected from 14 healthy

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Abbreviations used in this paper: ω -3 FA, omega 3 fatty acids; ATP, adenosine triphosphate; CD, Crohn's disease; DC, dendritic cell; DHA, docosahexaenoic acid; DSS, dextran sulfate sodium; ECAR, extracellular acidification rate; GPR, G protein-coupled receptor; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; IFN- γ , interferon gamma; IL, interleukin; IL10R, interleukin 10 receptor; KEGG, Kyoto Encyclopedia of Genes and Genomes; LP, lamina propria; mRNA, messenger RNA; OCR, oxygen consumption rate; RNA-seq, RNA sequencing; Th, T helper; TNF, tumor necrosis factor; Treg, regulatory T cell; UC, ulcerative colitis; UTMB, University of the Texas Medical Branch; WT, wild type.

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volunteers, and inflamed mucosa biopsy samples were obtained from 21 patients with active CD and 10 patients with active UC. Ethical approval was obtained from the institutional review board and ethics committee of the First Affiliated Hospital of Nanjing Medical University, and informed consent was obtained from all the participants. The characteristics of all human participants are included in [Supplementary Table 1](#).

Statistical Analysis

Data were analyzed by the 2-way Student *t* test, Mann-Whitney *U* test, 1-way analysis of variance (ANOVA) test, or Spearman correlation analysis using GraphPad Prism 9. All data are presented as mean \pm standard error of the mean, and a *P* value $< .05$ was considered statistically significant.

All other methods are included in the [Supplementary Materials](#).

RNA Sequencing Data

RNA sequencing (RNA-seq) data in this study are available in the Gene Expression Omnibus database under accession number GSE158782.

Results

Gpr120^{-/-} Mice Develop More Severe Colitis Upon Inflammatory Insult and Enteric Infection

To determine whether GPR120 regulates intestinal homeostasis, we induced colitis in WT and *Gpr120^{-/-}* mice by administering dextran sulfate sodium (DSS). First, we confirmed that *Gpr120* expression was knocked out in *Gpr120^{-/-}* mice ([Supplementary Figure 1A](#)). *Gpr120^{-/-}* mice showed more weight loss, suffered from more severe colitis, and produced more tumor necrosis factor (TNF) α and IL6 than WT mice ([Supplementary Figure 2A-D](#)). There were no differences in interferon gamma (IFN- γ) and IL17A production ([Supplementary Figure 2D](#)). However, IL10 was lower in colonic tissues of *Gpr120^{-/-}* mice than WT mice ([Supplementary Figure 2D](#)). There were no differences of IFN- γ^+ CD4 $^+$ T cells, IL17A $^+$ CD4 $^+$ T cells, and Foxp3 $^+$ Tregs in the colonic lamina propria (LP) between WT and *Gpr120^{-/-}* mice, but fewer intestinal IL10 $^+$ CD4 $^+$ T cells were present in *Gpr120^{-/-}* mice ([Supplementary Figure 2E-I](#)).

Similar results were obtained in WT and *Gpr120^{-/-}* mice infected with *Citrobacter rodentium* (*C. rodentium*), an enteric bacterial strain similar to IBD-associated human enteropathogenic *Escherichia coli*.²¹ *Gpr120^{-/-}* mice showed more weight loss than WT mice ([Supplementary Figure 2J](#)). Although there were no differences in the bacterial burdens at day 4, colony counts of fecal *C. rodentium* were higher in the *Gpr120^{-/-}* mice on day 7, and the difference was even more significant from day 10 ([Supplementary Figure 2K](#)). On day 14, *C. rodentium* was no longer detectable in WT mice, but counts were still high in *Gpr120^{-/-}* mice ([Supplementary Figure 2K](#)), suggesting the importance of GPR120 in adaptive immune responses against intestinal pathogens. When killed at 14 days postinfection, *Gpr120^{-/-}* mice developed more severe colitis and produced more TNF- α , IL6, and IFN- γ , but less IL10, in the colon than WT

mice ([Supplementary Figure 2L-N](#)). There were no differences in colonic IL17A production ([Supplementary Figure 2N](#)). These data indicate that GPR120 inhibits intestinal inflammation.

Gpr120^{-/-} CD4 $^+$ T Cells Induce More Severe Colitis

Although intestinal epithelial cells (IECs), DCs, and macrophages have been shown to express GPR120,^{16,20,22} its expression by CD4 $^+$ T cells has not been defined. We found that activated CD4 $^+$ T cells expressed GPR120 at a higher level than bone-marrow DCs and small bowel IECs, and GPR120 levels in activated CD4 $^+$ T cells were higher than in naive T cells ([Figure 1A](#) and [B](#)). In addition, Th1 cells, Th17 cells, and Treg cells expressed GPR120 at similar levels but higher than DCs and IECs ([Figure 1A](#) and [B](#)).

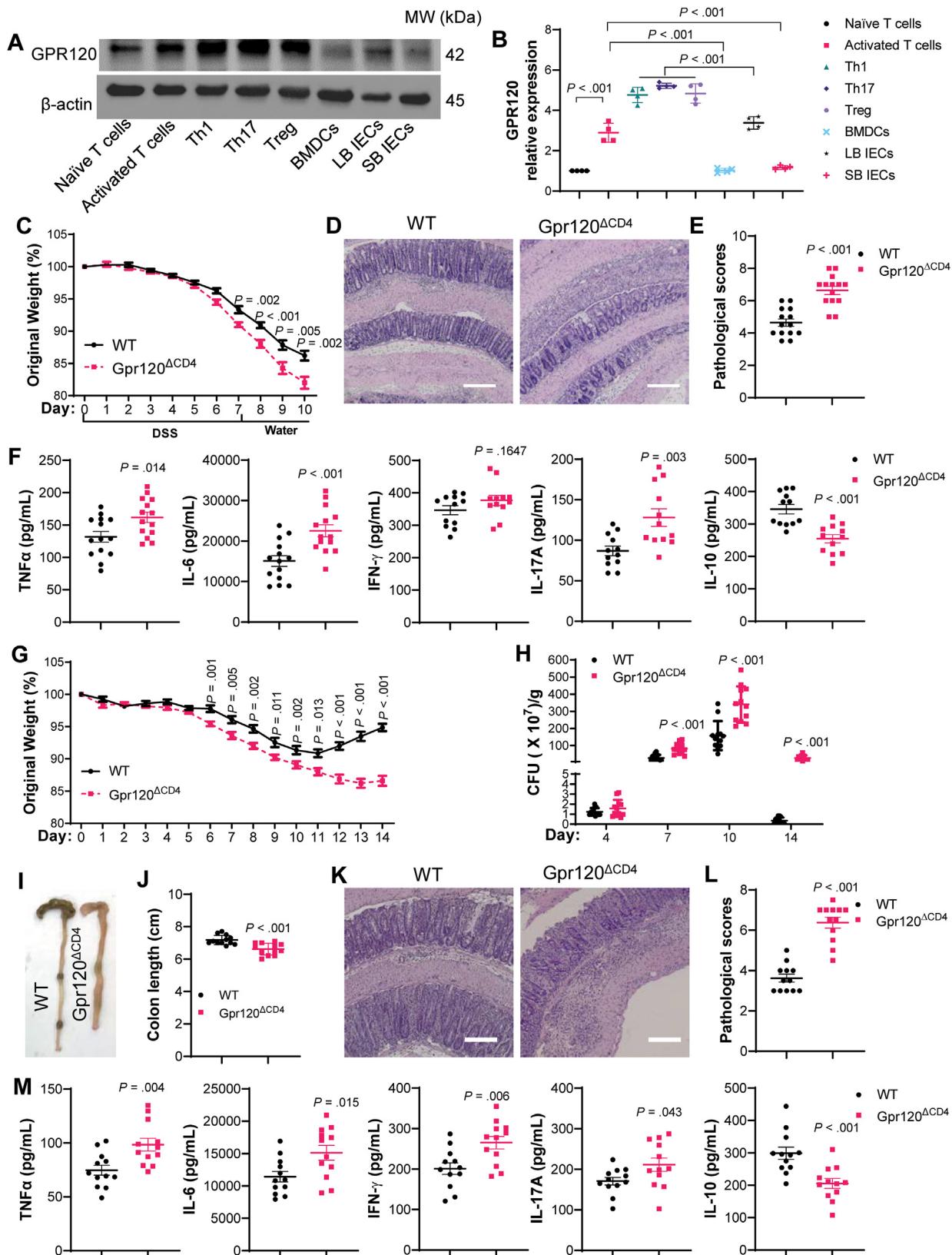
We next investigated the role of CD4 $^+$ T cell expression of GPR120 in intestinal homeostasis. First, we determined that *Gpr120* was specifically knocked out in CD4 $^+$ T cells from *Cd4^{cre}Gpr120^{fl/fl}* mice ([Supplementary Figure 1B](#) and [C](#)). We next induced colitis in *Cd4^{cre}Gpr120^{fl/fl}* mice and WT *Cd4^{cre}Gpr120^{fl/+}* mice using DSS. *Cd4^{cre}Gpr120^{fl/fl}* mice developed more severe colitis with more weight loss, had higher pathology scores in the colon, and produced more TNF- α , IL6, and IL17A, but less IL10, in the colon than WT mice ([Figure 1C-F](#)). There was no difference in IFN- γ ([Figure 1F](#)). LP IL10 $^+$ CD4 $^+$ T cells were decreased in *Cd4^{cre}Gpr120^{fl/fl}* mice, whereas there were no differences in IFN- γ^+ Th1 cells, IL17 $^+$ Th17 cells, and Foxp3 $^+$ Tregs ([Supplementary Figure 3A-E](#)), indicating that CD4 $^+$ T cell expression of GPR120 suppresses colitis, possibly through induction of IL10.

Next, we infected *Cd4^{cre}Gpr120^{fl/fl}* mice and WT *Cd4^{cre}Gpr120^{fl/+}* mice with *C. rodentium*. *Cd4^{cre}Gpr120^{fl/fl}* mice showed more weight loss compared with WT *Cd4^{cre}Gpr120^{fl/+}* mice ([Figure 1G](#)). Although there were no differences in the bacterial burdens at day 4, fecal *C. rodentium* levels were higher in *Cd4^{cre}Gpr120^{fl/fl}* mice than WT mice on days 7, 10, and 14 ([Figure 1H](#)). When mice were killed at 14 days postinfection, *Cd4^{cre}Gpr120^{fl/fl}* mice developed more severe colitis and produced more TNF- α , IL6, IFN- γ , and IL17A, but less IL10, in the colon ([Figure 1I-M](#)). More IFN- γ^+ Th1 cells and IL17A $^+$ Th17 cells, but fewer IL10 $^+$ T cells, were present in the LP of *Cd4^{cre}Gpr120^{fl/fl}* mice, whereas there was no difference in Foxp3 $^+$ Tregs ([Supplementary Figure 3F-J](#)).

To further verify the role of GPR120 in CD4 $^+$ T cells in regulating colitis, we used the CD4 $^+$ CD45RB hi T cell adoptive transfer model.²³ WT and GPR120-deficient CD4 $^+$ CD45RB hi T cells, isolated from WT *Cd4^{cre}Gpr120^{fl/+}* mice and *Cd4^{cre}Gpr120^{fl/fl}* mice, were transferred into *Rag^{-/-}* mice. The recipient mice were killed 6 weeks later. GPR120-deficient CD4 $^+$ CD45RB hi T cells induced more severe colitis in *Rag^{-/-}* mice ([Figure 2A-E](#)) and produced more TNF- α , IL6, IFN- γ , and IL17A, but less IL10, in the colon than did WT CD4 $^+$ CD45RB hi T cells ([Figure 2F](#)). Fewer intestinal IL10 $^+$ CD4 $^+$ T cells and more IFN- γ^+ Th1 cells and IL17A $^+$ Th17 cells were present in *Rag^{-/-}* mice

receiving GPR120-deficient T cells compared with recipient mice reconstituted with WT T cells, whereas there was no difference in Foxp3⁺ Tregs between the 2 groups

of mice (Figure 2G-K). These data indicate that GPR120 in CD4⁺ T cells is critical in regulating intestinal homeostasis.



GPR120 Agonist Promotes CD4⁺ T Cell Production of Interleukin 10 to Suppress Colitis

To investigate whether GPR120 indeed regulates CD4⁺ T cell functions, we cultured CD4⁺ T cells with or without a GPR120 agonist, CpdA,²⁰ for 2 days. Cells were collected, and the differences in the transcriptome were analyzed by RNA-seq. The GPR120 agonist altered the gene expression profile (Figure 3A and Supplementary Figure 4A), in which 1749 genes were up-regulated and 1709 genes were down-regulated (Supplementary Figure 4B). Gene Ontology functional enrichment analysis showed that the differentially expressed genes were enriched in various functions regarding metabolism, function, and biological processes, including the primary metabolic process, glycolytic process, regulation of immune system process, regulation of T cell-mediated immunity, regulation of T cell cytokine production, and cellular response to hypoxia (Supplementary Figure 4C). Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that the differentially expressed genes were enriched in 46 pathways, including inflammatory bowel disease (Figure 3B). CpdA promoted *Il10* but inhibited *Ifng* and *Il17* at the messenger RNA (mRNA) level, whereas it did not affect *Foxp3* expression (Figure 3C).

To verify the effect of CpdA on CD4⁺ T cell cytokine production at the protein level, we cultured CD4⁺ T cells with or without CpdA for 5 days to measure cytokine production using flow cytometry. CpdA promoted CD4⁺ T cell IL10 production, but it did not affect IFN- γ and IL17A under neutral conditions (Supplementary Figure 5A-F), which might be attributed to low protein expressions of IFN- γ and IL17A in CD4⁺ T cells under neutral conditions. GPR120 agonist promoted IL10 in CD4⁺ T cells under Th1 conditions (Figure 3D-F) but not under Th17 and Treg conditions (Supplementary Figure 5A and B). Furthermore, CD4⁺ T cells expressed higher levels of IL10 under Th1 conditions than under other polarization conditions (Figure 3D and E and Supplementary Figure 5A and B). Interestingly, CpdA promoted IFN- γ ⁺IL10⁺ T cells but not IFN- γ ⁺IL10⁻ T cells under Th1 conditions (Supplementary Figure 5G-J), indicating that CpdA renders self-regulatory function to Th1 effector cells. CpdA suppressed IL17A production under Th17 conditions (Supplementary Figure 5J and K), but it did not affect Treg differentiation (Supplementary Figure 5L and M). In addition, CpdA at the indicated dose did not affect CD4⁺ T cell viability and proliferation (Supplementary Figure 6A-C). To determine whether the natural ligand for

GPR120 also promotes IL10 production in CD4⁺ T cells, we treated CD4⁺ T cells with docosahexaenoic acid (DHA), a ω -3 FA, under Th1 conditions. As shown in Supplementary Figure 7A and B, DHA increased T cell IL10 production in a dose-dependent manner.

Because GPR120 promoted CD4⁺ T cell production of IL10, especially under Th1 conditions, and Th1 cells expressed higher levels of IL10, we next investigated whether GPR120 agonist-treated Th1 cells induced less severe colitis through induction of IL10. We treated WT and IL10-deficient CD4⁺ T cells with or without the GPR120 agonist CpdA for 5 days and then transferred them into *Rag*^{-/-} mice. The mice were killed 6 weeks later. The *Rag*^{-/-} recipient mice of CpdA-treated WT Th1 cells developed less severe colitis and produced less TNF- α , IL6, and IFN- γ , but more IL10, in the colon compared with *Rag*^{-/-} mice reconstituted with untreated WT Th1 cells (Figure 3G-J). However, the *Rag*^{-/-} mice receiving control IL10-deficient Th1 cells or CpdA-treated IL10-deficient Th1 cells developed severe colitis at similar levels with no differences in colonic cytokines (Figure 3G-J). We also used the anti-IL10R antibody to inhibit the IL10-IL10R pathway in *Rag*^{-/-} mice receiving CpdA-treated Th1 cells or control Th1 cells. Anti-IgG antibody was used as a control. CpdA-treated Th1 cells induced less severe colitis than control Th1 cells in *Rag*^{-/-} mice treated with control anti-IgG antibody (Supplementary Figure 8A-F). However, there were no differences in disease severity and cytokine production when the mice were treated with anti-IL10R antibody (Supplementary Figure 8A-F).

Because GPR120 did not affect Treg differentiation (Supplementary Figure 5L and M), we then investigated whether GPR120 affects Treg suppressive functions. CD45.1 WT CD4⁺CD45RB^{hi} T cells were transferred into *Rag*^{-/-} mice together with or without CD45.2 WT or GPR120-deficient Tregs, differentiated from naive CD4⁺ T cells of WT *Cd4*^{cre}*Gpr120*^{f/f} mice or *Cd4*^{cre}*Gpr120*^{f/f} mice. Both WT and GPR120-deficient Tregs suppressed colitis induced by CD4⁺CD45RB^{hi} T cells at similar levels (Supplementary Figure 9A-F). These data indicate that GPR120 does not affect Treg function in suppressing colitis.

Blimp1 Mediates GPR120 Induction of Interleukin 10 Through the mTOR-Stat3 Pathway

Next, we investigated the potential mechanisms underlying the GPR120 induction of IL10 in CD4⁺ T cells. RNA-seq

Figure 1. CD4⁺ T cell-specific GPR120-knockout mice develop more severe colitis upon DSS insult and *C. rodentium* infection. (A, B) GPR120 expression was measured in naive CD4⁺ T cells, activated CD4⁺ T cells, Th1 cells, Th17 cells, Tregs, bone marrow DCs, large bowel IECs, and small bowel IECs (n = 4/group). (A) Western blots and (B) GPR120 protein relative expression. (C-F) *Cd4*^{cre}*Gpr120*^{f/f} (WT) mice and *Cd4*^{cre}*Gpr120*^{f/f} (*Gpr120*^{ΔCD4}) mice (n = 14/group) were administrated with 1.65% DSS (weight/volume) in drinking water for 7 days, followed by drinking water alone for an additional 3 days. (C) Mouse weight change. (D) Representative intestinal H&E staining. (E) Pathology score. (F) Colonic secretion of cytokines. (G-M) *Cd4*^{cre}*Gpr120*^{f/f} (WT) mice and *Cd4*^{cre}*Gpr120*^{f/f} (*Gpr120*^{ΔCD4}) mice (n = 12/group) were orally infected with *C. rodentium* on day 0 and killed on day 14. (G) Weight change. (H) Fecal *C. rodentium* counts. (I) Representative gross morphology of the colon. (J) Colon length. (K) Representative intestinal H&E staining. (L) Pathology score. (M) Colonic secretion of cytokines. All data are pooled from 3 independent experiments. (D, K) Scale bar, 300 μ m. (A) One-way ANOVA, (C, F, G, H, J, M) unpaired Student *t* test, or (E, L) Mann-Whitney *U* test. CFU, colony-forming unit; LB, large bowel; MW, molecular weight; SB, small bowel.

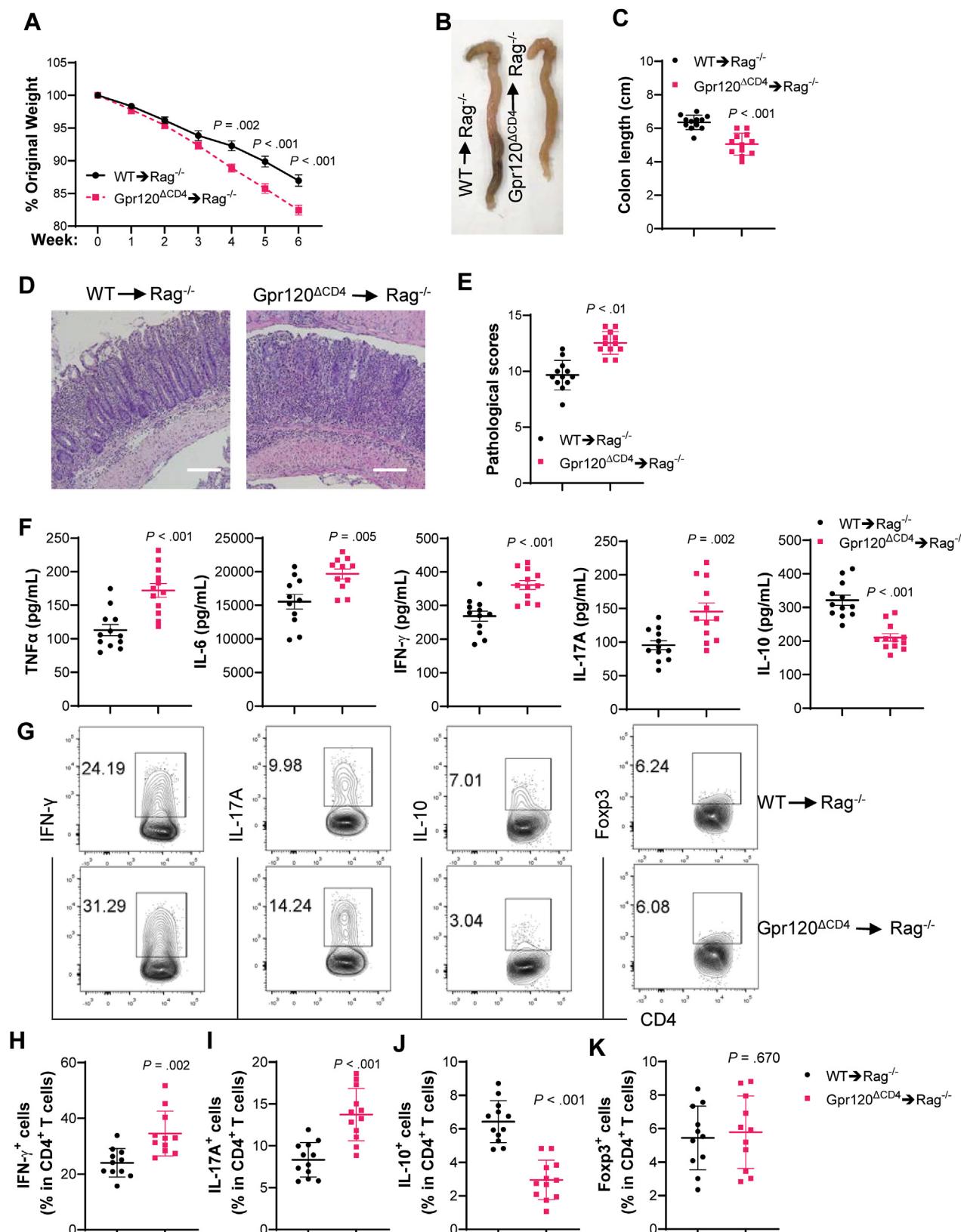
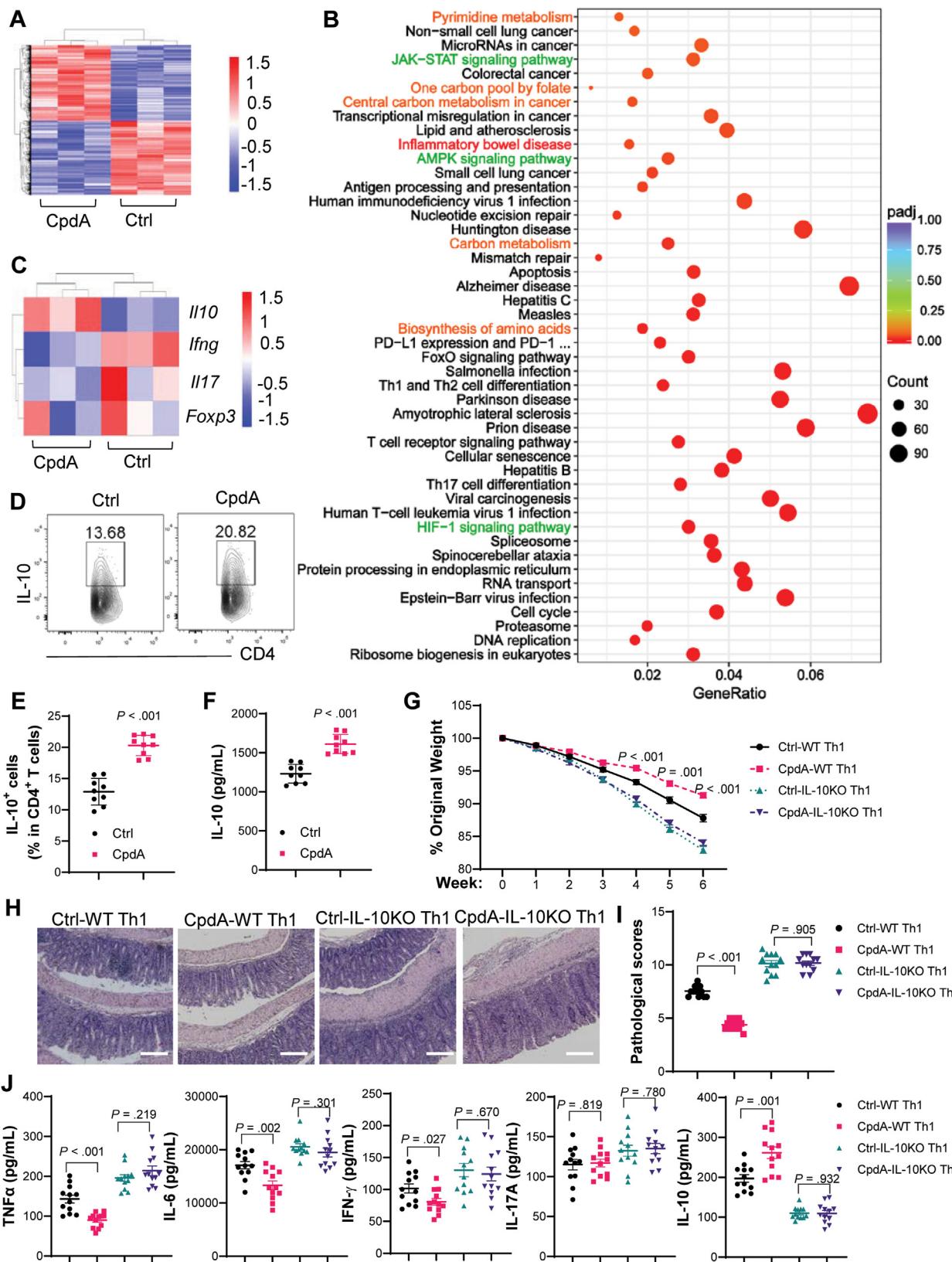


Figure 2. GPR120-deficient CD4⁺CD45RB^{hi} T cells induce more severe colitis in *Rag*^{-/-} mice. WT and GPR120-deficient CD4⁺CD45RB^{hi} T cells (1×10^5 cells/mouse) were intravenously transferred to *Rag*^{-/-} mice (n = 12/group). (A) Mouse weight changes. (B) Representative gross morphology of the colon. (C) Colon length. (D) Representative intestinal H&E staining. (E) Pathology score. (F) Colonic secretion of cytokines. (G) Representative flow cytometry profile of LP CD4⁺ T cells. (H–K) Bar charts of IFN γ^+ , IL17A $^+$, IL10 $^+$, and Foxp3 $^+$ CD4 $^+$ T cells. All data are pooled from 3 independent experiments. (D) Scale bar, 300 μ m. (A, C, F, H–K) Unpaired Student *t* test or (E) Mann-Whitney *U* test.

showed an increased tendency of transcription factors *Irf4*, *Prdm1* (encodes Blimp1), and *Maf* but a decreased expression of *Ahr* (Supplementary Figure 10A), all of which have

been implicated in regulating IL10 production in CD4⁺ T cells.^{24,25} To evaluate the transcriptome data, we treated CD4⁺ T cells with or without CpdA with increased sample



size. We found that CpdA significantly increased *Prdm1* expression (Figure 4A) but not *Irf4* and *Maf* (Supplementary Figure 10B and C). To determine if Blimp1 mediates GPR120 induction of IL10, we used *Cd4*^{cre}*Prdm1*^{f/f} mice, in which *Prdm1* was specifically knocked out in CD4⁺ T cells (Supplementary Figure 1D and E). We found that CpdA induction of IL10 was compromised in Blimp1-deficient CD4⁺ T cells (Figure 4B–D), indicating that Blimp1, at least partially, mediates GPR120 induction of IL10.

We then investigated how GPR120 regulates Blimp1 expression in CD4⁺ T cells. It has been reported that GPR120 activates mTOR in bone marrow-derived mesenchymal stem cells,²⁶ which has been shown to regulate IL10 production in T cells.²⁷ KEGG pathway enrichment analysis showed that GPR120 agonist altered the mTOR upstream AMP-activated protein kinase (AMPK) signaling pathway in T cells (Figure 3B). Treatment with GPR120 agonist CpdA promoted mTOR activation in CD4⁺ T cells (Figure 4E and F). Treatment with rapamycin, the mTOR inhibitor, decreased T cell expression of *Prdm1* (Figure 4G) and IL10 production induced by CpdA (Figure 4H and M–O). Given that Stat3 promotes Blimp1 expression and mTOR positively regulates Stat3^{28–30} and that RNA-seq data suggested an altered JAK-STAT signaling pathway in CpdA-treated T cells (Figure 3B), we hypothesized that GPR120 activation of mTOR induces IL10 production through the Stat3-Blimp1 pathway. Indeed, CpdA activated Stat3, which was inhibited by the mTOR inhibitor rapamycin (Figure 4I and J). Stattic, a selective Stat3 inhibitor, suppressed CpdA-induced *Prdm1* expression and IL10 production (Figure 4K–O). Furthermore, both mTOR and Stat3 inhibitors suppressed IL10 production induced by DHA (Supplementary Figure 7C and D). These data suggest that Blimp1 mediates GPR120 induction of IL10 through the mTOR-Stat3 pathway.

Blimp1 Is Essential for GPR120 Inhibition of T Effector Cell Induction of Colitis

We then investigated whether Blimp1 is essential for GPR120 inhibition of T effector cell induction of colitis. We treated Blimp1-deficient and WT CD4⁺ T cells from *Cd4*^{cre}*Prdm1*^{f/f} mice and *Cd4*^{cre}*Prdm1*^{f/+} mice with or without the CpdA for 5 days and then transferred them into *Rag*^{-/-} mice. *Rag*^{-/-} recipient mice of CpdA-treated WT Th1 cells developed less severe colitis and produced less TNF- α , IL6, and IFN- γ , but more IL10, in the colon than those with WT Th1 cells (Figure 4P–S). However, both CpdA-treated Blimp1-deficient Th1 cells and control Blimp1-deficient Th1 cells induced severe colitis and led to similar levels of

proinflammatory cytokines in the colon of *Rag*^{-/-} recipient mice (Figure 4P–S). Although intestinal IFN- γ ⁺CD4⁺ T cells were decreased and IL10⁺CD4⁺ T cells were increased in *Rag*^{-/-} mice reconstituted with CpdA-treated WT Th1 cells compared with the mice receiving control WT Th1 cells, similar levels of intestinal IL10⁺CD4⁺ T cells and IFN- γ ⁺CD4⁺ T cells were present in recipient mice receiving control Blimp1-deficient Th1 cells or CpdA-treated Blimp1-deficient Th1 cells (Supplementary Figure 11A, B, and E). All recipient mice showed similar levels of intestinal IL17⁺CD4⁺ T cells and Foxp3⁺ Tregs (Supplementary Figure 11A, C, and D).

Glycolysis Is Involved in GPR120 Induction of Interleukin 10 in CD4⁺ T Cells

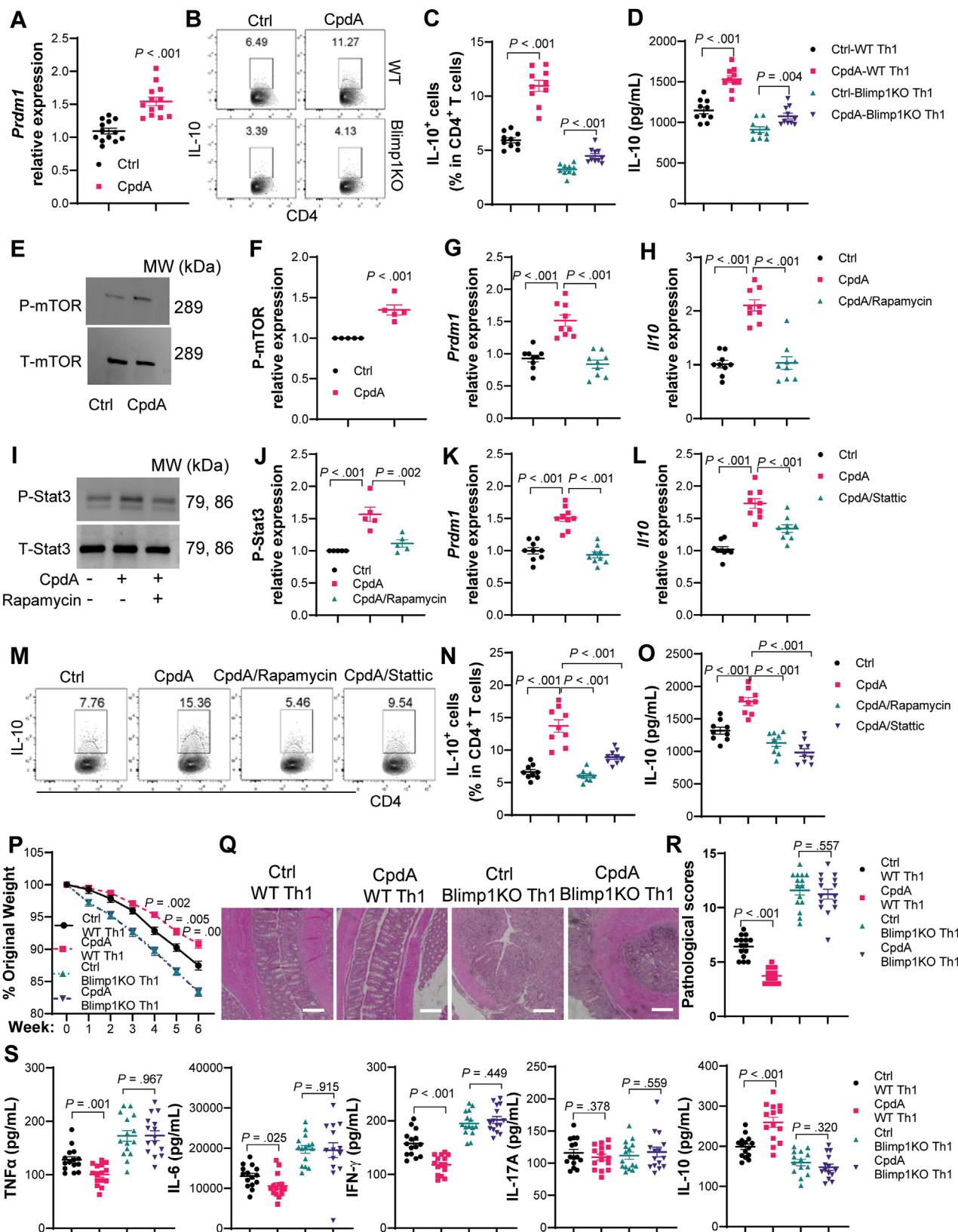
Because transcriptome data indicated that GPR120 affects the metabolism of CD4⁺ T cells (Figure 3B and Supplementary Figure 4C), we next investigated whether GPR120 modulates mitochondrial oxidation and glycolysis, the major events of metabolism, which differentially regulate T cell functions.³¹ First, we measured the oxygen consumption rate (OCR), primarily attributed to mitochondrial oxidation, and the extracellular acidification rate (ECAR), which represents glycolysis, in CD4⁺ T cells using an extracellular flux Seahorse analyzer. The GPR120 agonist CpdA enhanced OCR and ECAR levels in both naive T cells (Supplementary Figure 12A–D) and anti-CD3/anti-CD28-activated T cells (Supplementary Figure 12E–H). We also checked the key parameters of mitochondrial oxidation using the Mito stress test kit (Agilent, Santa Clara, CA). CpdA-treated CD4⁺ T cells exhibited enhanced OCR with higher basal respiration, adenosine triphosphate (ATP)-related respiration, maximal respiration, and spare respiratory capacity than control T cells (Figure 5A and B). To further assess the key parameters of glycolytic flux and exclude the non-glycolytic acidification, we measured ECAR levels in activated CD4⁺ T cells using the Glycolysis stress test kit (Agilent, Santa Clara, CA). CpdA promoted glycolysis and glycolytic capacity, but not glycolytic reserve, and slightly increased the ECAR levels related to the nonglycolytic activity (Figure 5C and D). In line with elevated glycolysis, CpdA promoted CD4⁺ T cell glucose uptake (Supplementary Figure 12I and J).

Glycolysis has been shown to regulate IL10 production in several types of immune cells.^{32,33} We then investigated whether GPR120-enhanced glycolysis contributes to up-regulated IL10 production in CD4⁺ T cells. We found that blocking glycolysis suppressed IL10 production induced by CpdA and DHA (Figure 5E–G and Supplementary Figure 7C

Figure 3. GPR120 promotes CD4⁺ T cell production of IL10 to suppress colitis. (A–C) Splenic CD4⁺ T cells were activated with anti-CD3/anti-CD28 with or without CpdA for 48 hours to analyze gene expression by RNA-seq ($n = 3$ /group). (A) Differentially expressed genes between CD4⁺ T cells treated with or without CpdA in a heatmap. Arbitrary units. (B) KEGG pathway enrichment analysis. (C) Specific gene expressions in a heatmap. Arbitrary units. (D–F) CD4⁺ T cells were activated with anti-CD3/anti-CD28 with or without CpdA under Th1 conditions ($n = 9$ /group). (D, E) Flow cytometry profile of IL10⁺CD4⁺ T cells after 5 days. (F) IL10 in culture supernatants after 2 days. (G–J) WT and IL10^{-/-}CD4⁺ T cells were activated with anti-CD3/anti-CD28 with or without CpdA under Th1 conditions for 5 days and transferred to *Rag*^{-/-} mice ($n = 12$ /group). (G) Mouse weight change. (H) Representative intestinal H&E staining. (I) Pathology score. (J) Colonic secretion of cytokines. (D–J) Data were pooled from 3 independent experiments. (H) Scale bar, 300 μ m. (E, F, G, J) Unpaired Student *t* test or (I) Mann-Whitney *U* test. Ctrl, control; KO, knockout; padj, adjusted *P* value.

and D). Additionally, inhibition of mTOR using rapamycin inhibited CpdA-induced glycolysis and glycolytic capacity (Figure 5H and I) and glucose uptake (Supplementary

Figure 12K and L), suggesting that GPR120 activation of mTOR regulates glycolysis. Glycolysis has been reported to promote HIF1 α expression,³⁴ which increases IL10



production in B cells.³³ KEGG pathway enrichment analysis suggested that GPR120 regulates the HIF1 signaling pathway in T cells (Figure 3B), and GPR120 agonist CpdA treatment promoted HIF1 α expression in CD4⁺ T cells (Supplementary Figure 10), which was suppressed by the glycolysis inhibitor 2-DG (Figure 5J). Furthermore, treatment with YC-1, a HIF1 α inhibitor, decreased CpdA-induced IL10 production (Figure 5K-M). Collectively, these data indicate that GPR120-elevated HIF1 α expression also contributes to GPR120 induction of IL10.

Oral Feeding of GPR120 Agonist Inhibits Colitis

Because the GPR120 agonist promoted CD4⁺ T cell production of IL10 and GPR120 regulates intestinal inflammation, we then investigated whether administering the GPR120 agonist could prevent and treat colitis. We first transferred WT CD4⁺CD45RB^{hi} T cells to *Rag*^{-/-} mice and gave the mice CpdA or carrier alone orally daily from the day of cell transfer until the mice were killed. Administering CpdA suppressed colitis, characterized by less weight loss, increased colon length, and decreased pathology scores, and inhibited TNF- α , IL6, IFN- γ , and IL17A, but promoted IL10 production, in the colon (Figure 6A-F). However, CpdA did not alleviate colitis severity in *Rag*^{-/-} recipients of GPR120-deficient CD4⁺CD45RB^{hi} T cells (Supplementary Figure 13A-F), suggesting that CD4⁺ T cells are indispensable in GPR120 regulation of colitis. Similar results were obtained in mice infected with *C. rodentium*, which were treated orally with or without CpdA daily for 10 days. The CpdA-treated mice developed less severe colitis with less weight loss, lower intestinal bacteria load, decreased pathology scores, and lower proinflammatory cytokines in the intestine than those treated with carrier alone (Supplementary Figure 14A-E). Administering CpdA promoted IL10 and IL22 production in the colon and increased intestinal expression of antimicrobial peptide regenerating islet-derived 3 gamma (Reg3 γ) (Supplementary Figure 14E and F). Furthermore, CpdA treatment decreased intestinal IFN- γ ⁺CD4⁺ T cells and IL17A⁺CD4⁺ T cells but increased intestinal IL10⁺CD4⁺ T cells and IL22⁺CD4⁺ T cells (Supplementary Figure 14 G-K). However, CpdA treatment did not affect the levels of intestinal Foxp3⁺ Treg (Supplementary Figure 14G and L).

Next, we investigated whether GPR120 agonist treats colitis. We transferred WT CD4⁺CD45RB^{hi} T cells into

Rag^{-/-} mice. Two weeks after cell transfer, when mice had developed mild colitis (Supplementary Figure 15), the mice were treated orally with or without CpdA daily for an additional 4 weeks. Administering CpdA inhibited colitis progression with decreased weight loss, increased colon length, lower pathology scores, and lower proinflammatory cytokine production, but increased IL10 production, in the intestine than carrier alone-treated mice (Figure 6 G-L). Collectively, these data suggest that GPR120 agonist prevents and treats colitis.

GPR120 Is Positively Correlated With Interleukin 10 in Patients With Inflammatory Bowel Disease

To investigate whether GPR120 is differentially expressed in the intestinal mucosa of patients with IBD, we retrieved the data from GSE11223 in the GEO database. *Gpr120* expression values were increased in the colonic mucosa of patients with UC compared with healthy control individuals (Figure 7A). The higher expression of intestinal GPR120 in patients with UC suggests the potential for oral GPR120 agonists as therapeutics in patients with IBD. Additionally, colonic *Gpr120* expression values were positively correlated with *Il10* in healthy control individuals, and it showed a tendency that *Gpr120* values were correlated with *Il10* in patients with UC (Figure 7B and C). To further verify the correlation between colonic GPR120 and IL10 expression, we collected colonic biopsy samples from 14 healthy control individuals, 10 patients with active UC, and 21 patients with active CD and measured GPR120 and IL10 expression. Consistent with the result retrieved from the database, *Gpr120* expression was positively correlated with *Il10* expression in colonic biopsy samples of healthy control individuals and patients with UC (Figure 7D and E). Patients with CD also showed the correlation between *Gpr120* and *Il10* in the mucosa (Figure 7F).

Discussion

Dietary regulation has been shown to play essential roles in health and diseases. GPR120, the receptor for ω -3 FA, has been implicated in regulating metabolic diseases and several types of immune cells.^{16,19} However, the role of GPR120 in the regulation of intestinal inflammation is

Figure 4. Blimp1 mediates GPR120 induction of IL10 through the mTOR-Stat3 pathway. Splenic CD4⁺ T cells were activated with anti-CD3/anti-CD28 with or without CpdA and various inhibitors under Th1 conditions. (A) *Prdm1* mRNA expression on day 2 ($n = 13$ /group). (B, C) Flow cytometry profile of IL10⁺CD4⁺ T cells in WT and Blimp^{-/-}CD4⁺ T cells on day 5. (D) IL10 in culture supernatants on day 2 ($n = 10$ /group). (E, F) Phosphorylated and total mTOR expressions at 30 minutes ($n = 5$ /group). (G) *Prdm1* and (H) *Il10* mRNA expression in CD4⁺ T cells treated with or without rapamycin on day 2 ($n = 9$ /group). (I, J) Phosphorylated and total Stat3 expressions at 2 hours in CD4⁺ T cells treated with or without CpdA with or without rapamycin ($n = 5$ /group). (K) *Prdm1* and (L) *Il10* mRNA expression in CD4⁺ T cells treated with or without CpdA with or without Stat3 ($n = 9$ /group). (M, N) Flow cytometry profile of IL10⁺CD4⁺ T cells on day 5 and (O) IL10 in culture supernatants on day 2 ($n = 9$ /group). (P-S) WT and Blimp^{-/-} CD4⁺ T cells from *Cd4*^{cre}*Prdm1*^{f/f} mice and *Cd4*^{cre}*Prdm1*^{t/t} mice were activated with anti-CD3/anti-CD28 with or without CpdA under Th1 conditions for 5 days and transferred to *Rag*^{-/-} mice ($n = 15$ /group). (P) Mouse weight change. (Q) Representative intestinal H&E staining. (R) Pathology score. (S) Colonic secretion of cytokines. All data are pooled from 3 independent experiments. (A, C, D, F, J, P, S) Unpaired Student *t* test, (G, H, N, O) 1-way ANOVA, or (R) Mann-Whitney *U* test. P-mTOR, phosphorylated mTOR; T-mTOR, total m-TOR.

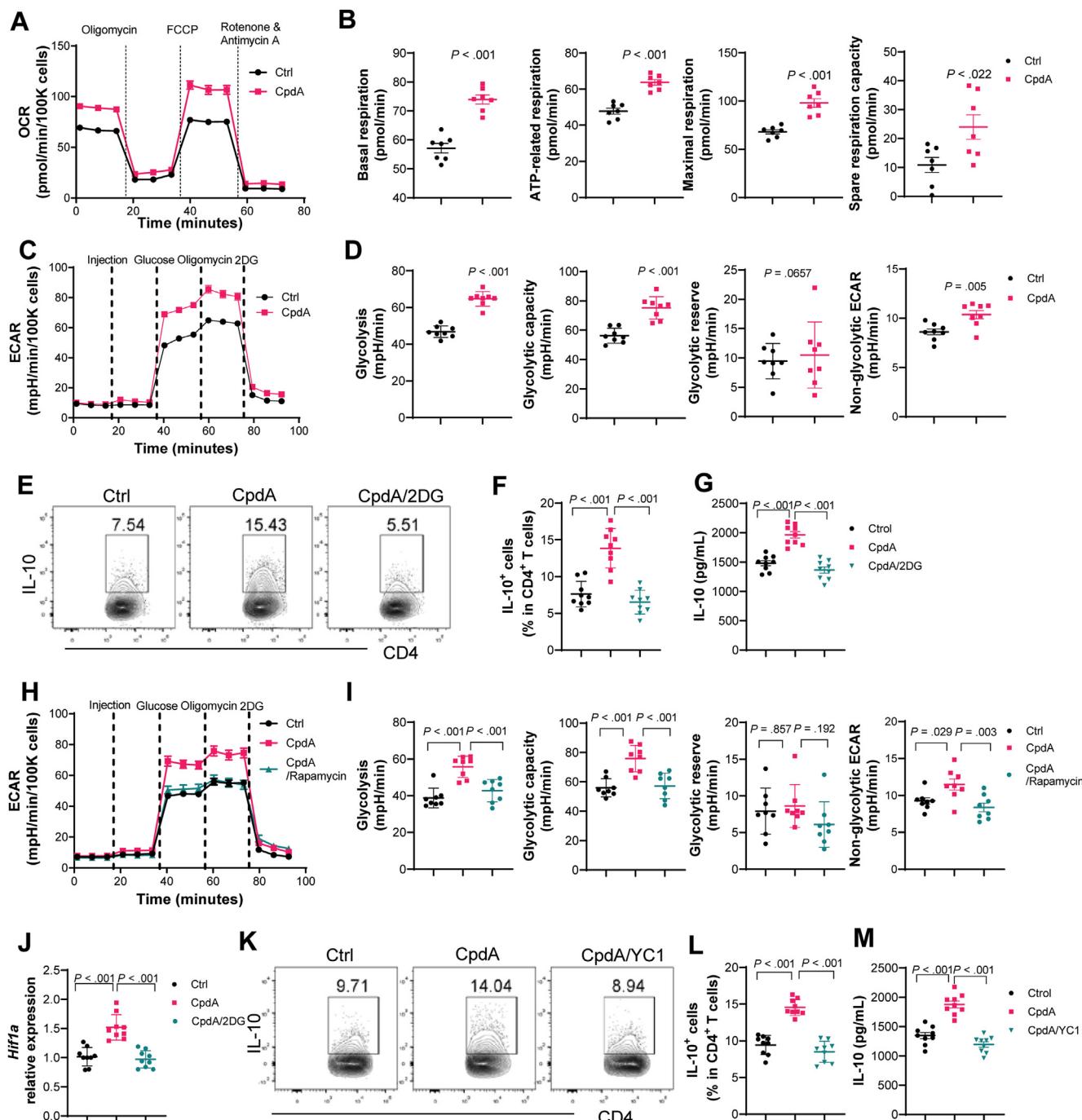
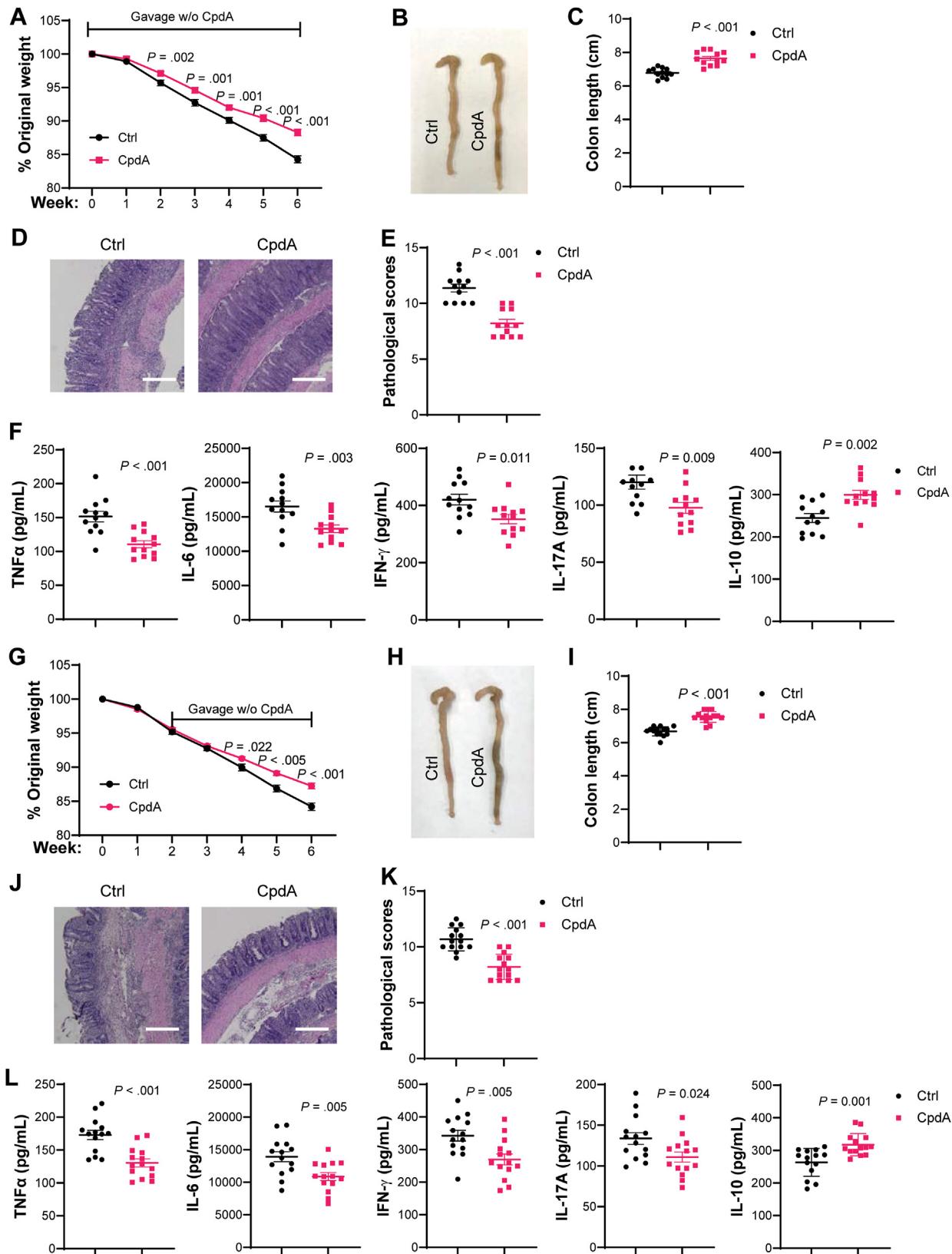


Figure 5. GPR120 agonist promotes IL10 production through the glycolysis-HIF1 α pathway. (A, B) Splenic CD4 $^{+}$ T cells were activated with anti-CD3/anti-CD28 with or without CpdA under Th1 conditions for 48 hours, and the Mito stress test kit was used to measure the parameters of mitochondrial respiration ($n = 7$ /group). The OCR profile, basal respiration, ATP-related respiration, maximum respiration, and spare respiration capacity. (C, D, H, I) Splenic CD4 $^{+}$ T cells were activated with anti-CD3/anti-CD28 under Th1 conditions for 48 hours, and a Glycolytic stress test kit was used to measure the key parameters of glycolysis ($n = 8$ /group). The ECAR profile, glycolysis, glycolytic capacity, glycolytic reserve, and nonglycolytic ECAR. (E–G) CD4 $^{+}$ T cells were treated with or without CpdA with or without 2-DG ($n = 9$ /group). (E, F) IL10 $^{+}$ CD4 $^{+}$ T cells on day 5 and (G) IL10 in culture supernatants on day 2. (J) Hif1 α mRNA expression in CD4 $^{+}$ T cells treated with or without CpdA with or without 2-DG at 48 hours ($n = 9$ /group). (K–M) CD4 $^{+}$ T cells were treated with or without CpdA with or without YC-1 ($n = 9$ /group). (K, L) Flow cytometry profile of IL10 $^{+}$ CD4 $^{+}$ T cells at day 5 and (M) IL10 production in culture supernatants on day 2. (E–G, J–M) Data are pooled from 3 independent experiments. (B, D) Unpaired Student *t* test or (F, G, I, L, M) 1-way ANOVA. FCCP, carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone.

unknown. In this study, we showed that GPR120 promotes CD4⁺ T cell production of IL10 by up-regulating Blimp1 and enhancing glycolysis to inhibit colitis, thus providing novel

insights into a critical role for GPR120 in maintaining intestinal homeostasis through the modulation of CD4⁺ T cell functions.



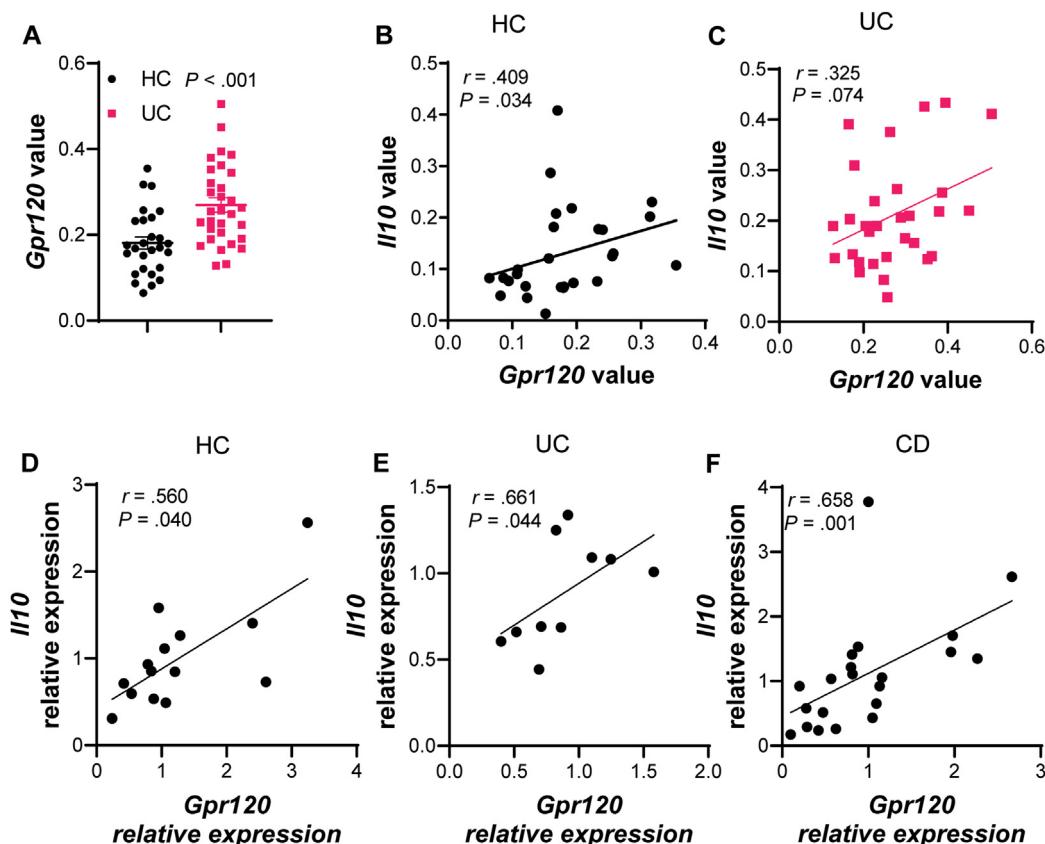


Figure 7. *Gpr120* is positively correlated with *II10* in human colonic mucosa. (A–C) The data from GSE11223 in the Gene Expression Omnibus database were retrieved. (A) *Gpr120* expression in the colonic mucosa of healthy control individuals (HC, n = 27) and patients with UC (n = 31). (B, C) The correlation between colonic *Gpr120* and *II10* in (B) healthy control individuals and (C) patients with UC. (D–F) Intestinal biopsy samples were collected from healthy control individuals (n = 14), patients with UC (n = 10), and patients with CD (n = 21). The correlation between intestinal *Gpr120* and *II10* in (D) healthy control individuals, (E) patients with UC, and (F) patients with CD. (A) Unpaired Student *t* test or (B–F) Spearman correlation. HC, healthy control individuals.

Dietary ω -3 FA are beneficial for preventing metabolic syndrome and inflammation.¹² As a receptor of ω -3 FA, GPR120 regulates insulin signaling and inflammation in adipose tissue and controls the energy balance.^{17,19} GPR120 agonist increases insulin sensitivity, indicating that GPR120 might be a promising target for diabetes.^{16,20,35} The intestinal tract directly interacts with dietary components, and several intestinal disorders have been linked to diet.¹⁴ Nevertheless, the exact role of diet in IBD pathogenesis remains poorly understood. Consumption of ω -3 FA is negatively associated with IBD incidence.¹⁵ We found that DHA, a ω -3 FA, promoted T cell production of IL10. It has been reported that dietary intake of ω -3 FA increased the blood levels of ω -3 FA,³⁶ suggesting that

dietary ω -3 FA could be absorbed in the intestine and transferred into circulation to regulate circulating CD4 $^{+}$ T cell production of IL10. IL10 $^{+}$ CD4 $^{+}$ T cells could be recruited to the inflamed intestines to suppress colitis. It is also possible that dietary ω -3 FA might directly affect CD4 $^{+}$ T cell functions in the gut.

GPRs are actively involved in most physiologic responses in humans and have become one of the successful targets of pharmaceuticals.³⁷ Previous studies have demonstrated the potential treatment of the GPR120 agonist for treating diabetes and metabolic syndrome.^{16,20,35,37} We showed that *Gpr120* $^{-/-}$ mice developed more severe colitis upon inflammatory insult and enteric infection and that oral administration of the GPR120 agonist prevented and treated

Figure 6. Oral feeding of GPR120 agonist prevents and treats colitis. (A–F) WT CD4 $^{+}$ CD45Rb hi T cells (1×10^5 cells/mouse) were intravenously transferred to *Rag* $^{-/-}$ mice and the mice were administered CpDA (20 mg/kg) or carrier alone orally (n = 12/group) daily from the day of cell transfer until the mice were killed 6 weeks after cell transfer. (A) Mouse weight change. (B) Gross morphology of the colon. (C) Colon length. (D) Representative intestinal H&E staining. (E) Pathology score. (F) Colonic secretion of cytokines. (G–L) WT CD4 $^{+}$ CD45Rb hi T cells (1×10^5 cells/mouse) were intravenously transferred to *Rag* $^{-/-}$ mice and the mice were administered CpDA (20 mg/kg) or carrier alone orally (n = 12/group) daily from 2 weeks after cell transfer for an additional 4 weeks. (G) Mouse weight change. (H) Gross morphology of the colon. (I) Colon length. (J) Representative intestinal H&E staining. (K) Pathology score. (L) Colonic secretion of cytokines. All data are pooled from 2 independent experiments. (D, J) Scale bar, 300 μ m. (A, C, F, G, I, L) Unpaired Student *t* test or (E, K) Mann-Whitney *U* test.

colitis, indicating that GPR120 agonists might be potential therapeutic targets for IBD.

Excessive CD4⁺ T cell responses have been considered critical in driving intestinal inflammation, whereas IL10 restricts proinflammatory responses to maintain intestinal homeostasis. IL10 produced by CD4⁺ effector T cells exerts a self-limiting mechanism to prevent an exaggerated T cell response in the intestines, which otherwise would be detrimental.³⁸ Although several types of cells, including IECs, macrophages, and adipocytes, have been shown to express GPR120, it was unknown whether CD4⁺ T cells express GPR120. We showed that activated CD4⁺ T cells express GPR120 at high levels and that CD4⁺ T cell-specific GPR120-knockout mice developed more severe colitis. Furthermore, transfer of GPR120-deficient CD4⁺CD45Rb^{hi} T cells induced more severe colitis than WT CD4⁺CD45Rb^{hi} T cells with lower levels of IL10 in the intestine. GPR120 agonist treatment inhibited WT, but not IL10-deficient, T cell induction of colitis, indicating that GPR120 promotes T cell IL10 production to inhibit colitis development. It has been shown that the lack of IL10 in CD4⁺ T cells worsens colitis but does not affect or slightly increases the clearance of *C. rodentium*.^{39,40} In our study, GPR120 agonist also increases IL22 production and Reg3 γ expression in the intestine, which could promote the intestinal clearance of *C. rodentium*.⁴¹ Meanwhile, higher levels of IL10 suppress excessive inflammation. The combination of IL10 and IL22 might contribute to the decreased severity of colitis and enhanced *C. rodentium* clearance. Variants in *Prdm1*, which encodes Blimp1, have been associated with the susceptibility of developing IBD.⁴² Blimp1 plays an essential role in promoting IL10 production in CD4⁺ T cells.⁴³ We showed that GPR120 agonist inhibited WT but not Blimp^{-/-} Th1 cell induction of colitis with higher levels of IL10 production, indicating that Blimp1 mediates GPR120 regulation of colitis through IL10 production.

Different CD4⁺ T cells require distinct metabolic programs compatible with their functional demands. Although quiescent CD4⁺ T cells are characterized by mixed-fuel oxidative phosphorylation, activated T cells become more glycolytic with increased oxidative phosphorylation for fulfilling the requirement of rapid cell growth and proliferation.³¹ Metabolic programs have been shown to involve and regulate CD4⁺ T cell functions. However, how the metabolic pathways affect T cell production of IL10 has not been fully defined. Our study found that the GPR120 agonist increased CD4⁺ T cell oxygen consumption and glycolysis, leading to higher ATP production and energy levels. Blocking glycolysis compromised GPR120-induced IL10 production, indicating that enhanced glycolysis mediates GPR120 promotion of IL10 production in CD4⁺ T cells. Additionally, the GPR120 agonist increased T cell expression of HIF1 α expression. Although HIF1 α has been reported to suppress mitochondrial respiration,⁴⁴ the increased oxidation in CD4⁺ T cells might be attributed to other pathways affected by GPR120. A recent report showed that distinct mitochondrial metabolism determines CD4⁺ T cell differentiation and function⁴⁵; thus, it will be interesting to investigate whether the mode of

mitochondrial metabolism is also involved in GPR120 induction of IL10 in T cells.

In summary, our study demonstrated that GPR120 suppresses CD4⁺ T cell induction of colitis through promoting IL10 production. Oral administration of the GPR120 agonist inhibits colitis, which presents GPR120 as a potential therapeutic target for IBD treatment.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://doi.org/10.1053/j.gastro.2021.09.018>.

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Conflicts of interest

The authors disclose no conflicts.

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