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Activity of SHIP, Which Prevents Expression of Interleukin 1β, Is Reduced in Patients With Crohn's Disease

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BACKGROUND & AIMS: Crohn's disease (CD) is associated with a dysregulated immune response to commensal microorganisms in the intestine. Mice deficient in inositol polyphosphate 5′-phosphatase D (INPP5D, also known as SHIP) develop intestinal inflammation resembling that of patients with CD. SHIP is a negative regulator of PI3Kp110α activity. We investigated mechanisms of intestinal inflammation in *Inpp5d*$^{-/-}$ mice (SHIP-null mice), and SHIP levels and activity in intestinal tissues of subjects with CD. **METHODS:** We collected intestines from SHIP-null mice, as well as *Inpp5d*$^{+/+}$ mice (controls), and measured levels of cytokines of the interleukin 1 (IL1) family (IL1α, IL1β, IL1ra, and IL6) by enzyme-linked immunosorbent assay. Macrophages were isolated from lamina propria cells of mice, IL1β production was measured, and mechanisms of increased IL1β production were investigated. Macrophages were incubated with pan–phosphatidylinositol 3-kinase inhibitors or PI3Kp110α-specific inhibitors. Some mice were given an antagonist of the IL1 receptor; macrophages were depleted from ilea of mice using clodronate-containing liposomes. We obtained ileal biopsies from sites of inflammation and peripheral blood mononuclear cells (PBMCs) from treatment-naïve subjects with CD or without CD (controls), and measured SHIP levels and activity. PBMCs were incubated with lipopolysaccharide and adenosine triphosphate, and levels of IL1β production were measured. **RESULTS:** Inflamed intestinal tissues and intestinal macrophages from SHIP-null mice produced higher levels of IL1B and IL18 than intestinal tissues from control mice. We found PI3Kp110α to be required for macrophage transcription of *Il1b*. Macrophage depletion or injection of an IL1 receptor antagonist reduced ileal inflammation in SHIP-null mice. Inflamed ileal tissues and PBMCs from patients with CD had lower levels of SHIP protein than controls ($P < .0001$ and $P < .0002$, respectively). There was an inverse correlation between levels of SHIP activity in PBMCs and induction of IL1β production by lipopolysaccharide and adenosine triphosphate ($R^2 = .88$). **CONCLUSIONS:** Macrophages from SHIP-deficient mice have increased PI3Kp110α-mediated transcription of *Il1b*, which contributes to spontaneous ileal inflammation. SHIP levels and activity are lower in intestinal tissues and peripheral blood samples from patients with CD than controls. There is an inverse correlation between SHIP activity and induction of IL1β production by lipopolysaccharide and adenosine triphosphate in PBMCs. Strategies to reduce IL1B might be developed to treat patients with CD found to have low SHIP activity.

Keywords: PI3Kp110α; Signal Transduction; Inflammatory; Phosphatidylinositol.

Crohn's disease (CD) is a subtype of inflammatory bowel disease (IBD) characterized by chronic inflammation along the gastrointestinal tract.[1] IBD affects 1 in 150 people in North America and the incidence of disease is increasing in developed countries.[1,2] Although the etiology of disease remains unknown, current thinking is that CD occurs in genetically susceptible individuals due to an inappropriate immune response to intestinal flora.[3,4] Biological therapy, monoclonal antibodies directed against tumor necrosis factor (TNF)α, is effective at inducing remission and has revolutionized the treatment for CD.[5] However, some patients are refractory to biological therapy or therapy becomes ineffective because patients develop antibodies against the drug.[6,7]

The pro-inflammatory cytokine, interleukin (IL) 1β, plays a critical role in IBD pathogenesis. IL1β is secreted from intestinal tissues and macrophages isolated from patients with IBD, and IL1β levels correlate with disease severity.[8–11] IL1β acts as an alarm cytokine, initiating the inflammatory response, thus its production is tightly regulated by a 2-step process: (1) Toll-like receptor (TLR) or endogenous ligands induce *IL1B* transcription, which is translated to pro–IL1β, an inactive precursor; (2)

Abbreviations used in this paper: **ATP, adenosine triphosphate; BMM, bone marrow–derived macrophage; CD, Crohn's disease; clod-lip, clodronate-containing liposomes; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; PI3K, phosphatidylinositol 3-kinase; SHIP, SH2 domain-containing inositolphosphate 5′-phosphatase; TLR, Toll-like receptor; TNF, tumor necrosis factor; Wm, wortmannin.**

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numerous stimuli, typically danger-associated molecular patterns, cause assembly of the inflammasome, a hetero-oligomeric protein complex, which catalyzes the processing of pro−IL1β for secretion.[12–14] IL1β can feedback and activate cells to induce *IL1B* transcription. Hence, monogenic gain-of-function mutations leading to increased IL1β production cause a group of autoinflammatory diseases (periodic fever syndromes), which can be treated with anakinra, an IL1 receptor antagonist.[1,12] Intestinal inflammation is a common complication of monogenic autoinflammatory diseases and primary immune deficiencies characterized by increased IL1β production.[1,15–17] A gene variant in *ATG16L1* (rs2241880), which has been associated with CD and high IL1β production, and a second intronic gene variant (rs12994997), which has traditionally been associated with ATG16L1, are located adjacent to the human gene encoding SH2 domain-containing inositolphosphate 5′-phosphatase (SHIP), *INPP5D*, and may impact disease by affecting *ATG16L1* and/or *INPP5D*.[18–20] IL1β antagonism has been used effectively to treat some genetically defined forms of very early onset IBD[21] and may be more broadly applicable for the treatment of subgroups of IBD.

Phosphatidylinositol 3-kinase (PI3K) is critical in cellular processes, including growth, differentiation, proliferation, and inflammation.[22] Class I PI3Ks are heterodimeric enzymes composed of a regulatory subunit; class IA contains a catalytic subunit, p110α, p110β, or p110δ, and class IB contains the p110γ catalytic subunit.[22] PI3Kp110 catalytic subunits have overlapping and unique functions downstream of different receptors.[23] SHIP is a hematopoietic-specific negative regulator of class I PI3K. SHIP antagonizes PI3K activity by dephosphorylating the PI3K-generated second messenger, PI(3,4,5)P_3.[24] Myeloid cells from SHIP-deficient mice are hyperproliferative[25] and hyperresponsive to growth factor, immune, and inflammatory stimuli.[26–30]

We, and others, have reported that SHIP$^{-/-}$ mice develop spontaneous CD-like intestinal inflammation.[31,32] We investigated the cause of intestinal inflammation in SHIP$^{-/-}$ mice to determine the contribution of SHIP$^{-/-}$ macrophages to pathology and to validate these findings in CD patients. We report that ileal macrophages from SHIP$^{-/-}$ mice produce high levels of IL1β caused by increased class I PI3Kp110α-driven *Il1b* transcription. Macrophage depletion or treatment with anakinra, an IL1 receptor antagonist, reduced ileal inflammation in SHIP$^{-/-}$ mice. Human subjects with CD had decreased SHIP protein levels and activity in ileal biopsies despite increased numbers of SHIP-expressing immune cells in tissues. Subjects with CD also had decreased SHIP activity in their PBMCs, which inversely correlated with the ability of their PBMCs to produce IL1β.

Materials and Methods

Descriptions of methods described previously are included in the Supplementary Material.

Mice

Mice heterozygous for SHIP expression (*Inpp5d*$^{+/-}$) on a mixed C57BL/6×129Sv background (F2 generation) were used to generate SHIP$^{+/+}$ and SHIP$^{-/-}$ littermates for experiments.[31] Mice used for experiments were between 4 and 8 weeks of age. Mice were housed in the Animal Research Centre at the Child & Family Research Institute, which is specific-pathogen free. Experiments were performed in accordance with Canadian Council on Animal Care guidelines (protocol numbers A09-0027 and A09-0032).

Macrophage Derivation and Isolation

Bone marrow macrophages (BMMs) were derived from BM aspirates of femura and tibiae from SHIP$^{+/+}$ and SHIP$^{-/-}$ mice, as described previously.[28] Ileal macrophages were prepared from lamina propria cells and selected using the mouse monocyte enrichment kit (StemCell Technologies, Vancouver, BC, Canada), as described previously.[33] Macrophage populations were ≥95% F4/80^{+}Mac-1^{+}.

Cell Stimulations

Cells were plated at a density of 0.5 × 10^6 cells/mL and stimulated with 10 ng/mL lipopolysaccharide (LPS) (*Eschericia coli* serotype 127:B8, Sigma-Aldrich, St Louis, MO) for 5 hours, 5 mM adenosine triphosphate (ATP) for 1 hour, or LPS for 5 hours +ATP for the final 1 hour. Cell supernatants were harvested and clarified by centrifugation. Inhibitors were added to cultures 30 minutes before addition of LPS or ATP. Commercially available inhibitors, controls, and final concentrations of each were: glybenclamide (100 μM; Sigma-Aldrich, St Louis, MO), Z-YVAD-fmk (40 μM; Sigma-Aldrich), LY303511 (14 μM; Calbiochem, San Diego, CA), LY294002 (14 μM; Calbiochem), dimethyl sulfoxide (0.1%), and wortmannin (Wm; 50 nM; Calbiochem). Isoform-specific PI3K inhibitors were synthesized as described previously and used at a concentration of 10 μM.[30,34]

Cytokine Measurements

Cytokine measurements were performed on clarified full-thickness tissue homogenates from mice or supernatants using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions. ELISA kits for mouse IL1α, IL1β, IL1ra, and IL6, and human IL1β were from BD Biosciences (Mississauga, ON, Canada); the IL18 ELISA kit was from MBL International (Woburn, MA).

Gene Expression Analysis

RNA was prepared from mouse tissue or cells using the NucleoSpin RNA II Total RNA Isolation Kit (Macherey-Nagel, Bethlehem, PA) and reverse transcribed using Superscript II (Invitrogen, Burlington, ON). Gene expression was measured by quantitative polymerase chain reaction using the AB Applied Biosystems Taqman Universal Master Mix II (Life Technologies, Burlington, ON). IL1β (*Il1b*) gene expression was normalized to ribosomal protein RPLP0 (*Rplp0*). Primer/probe sequences are in Supplementary Material.

Macrophage Depletion and Anakinra Treatment

Macrophages were depleted from mouse ilea using clodronate-containing liposomes (clod-lip), as described previously.[35,36]

Anakinra was injected intraperitoneally into mice daily at a dose of 150 mg/kg. Mice were treated either prophylactically

(from 4−6 weeks of age), before the onset of inflammation, or therapeutically (from 6−8 weeks of age), after the establishment of inflammation. An equal volume of phosphate-buffered saline was injected into mice as a control.

Sircol Assays

Sircol assays were performed as described previously.[31]

Subjects With Crohn's Disease and Control Subjects

Experiments were performed in accordance with ethical guidelines and with approval by the University of British Columbia Research Ethics Boards (protocol number H09-01826). Subjects seen in the Division of Gastroenterology at BC Children's Hospital were recruited into the study. No subjects had been previously diagnosed with, or treated for, IBD or other inflammatory pathology. Four ileal and 4 colonic biopsies were taken from sites of inflammation that were adjacent to tissues harvested for pathologic assessment in subjects with CD. Biopsies were taken from comparable, uninflamed sites in subjects, who were not subsequently diagnosed with IBD. Peripheral blood was taken from the site of intravenous insertion during colonoscopy. Diagnosis of CD with ileal inflammation or no disease was based on pathologic assessment and colonoscopy. Eight subjects diagnosed with ileal CD (no colonic involvement) and 14 subjects, who did not have IBD, were included in analyses.

Biopsies were fixed for H&E staining or used immediately for analyses. PBMCs were isolated by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare, Piscataway, NJ). PBMCs were washed and resuspended at 0.5×10^6 cells/mL in Iscove's modified Dulbecco's medium/10% fetal bovine serum for assays.

SHIP Activity Assays

SHIP was immunoprecipitated with an anti-human SHIP1 antibody (N-1, sc6244; Santa Cruz Biotechnology, CA) and assayed as described previously.[30]

Histologic Analyses

Biopsies from human subjects and ilea from $SHIP^{+/+}$ and $SHIP^{-/-}$ mice were fixed in phosphate-buffered saline–buffered 10% formalin at 4°C for 24 hours. Tissue sections were embedded in paraffin, and 5 μm cross-sections were cut and stained with H&E or Masson's trichrome, as per manufacturer's instructions (Sigma-Aldrich). Images were acquired using a Zeiss Axiovert 200 microscope, AxiocamHR camera, and Axiovision 4.0 software. Immune cell infiltrates were counted at 20× magnification in 6 H&E-stained sections separated by ≥50 μm. Crypt/villus length (mouse) was determined by counting epithelial cell nuclei from the crypt base to the villus tip on uniform horizontal ileal cross-sections. Representative crypt/villi (10/section) were counted in 6 H&E-stained ileal sections for each mouse. Thickness of the muscularis externa was measured at 6 points in 10 cross-sections of the ileum separated by ≥50 μm. Counting and measurements were performed by 2 individuals blinded to experimental condition.

For macrophage staining, slides were mounted and stained with F4/80, as described previously.[35] Macrophages were counted at 20× magnification at 6 points in 6 H&E-stained sections separated by ≥50 μm by 2 individuals blinded to experimental condition.

For detection of active caspase-1, F4/80 stained slides were co-stained with YVAD-FLICA immediately before counterstaining with Harris' hematoxylin, according to manufacturer's instructions (ImmunoChemistry Technologies, Bloomington, MN). Tissue sections were thoroughly rinsed and then stained with 4′,6-diamidino-2-phenylindole (Invitrogen, Carlsbad, CA).

Statistical Analyses

Unpaired 2-tailed Student's t tests were performed using GraphPad Prism software (version 5; GraphPad Software, Inc, La Jolla CA), with the Bonferroni correction for multiple comparisons. Differences were considered significant at $P < .05$.

Results

Ileal Macrophages From $SHIP^{-/-}$ Mice Produce High Levels of Interleukin 1β and Interleukin 18

We recently reported that $SHIP^{-/-}$ mice develop spontaneous CD-like intestinal inflammation.[31] Full-thickness tissue homogenates from $SHIP^{-/-}$ mice did not have elevated levels of proinflammatory cytokines TNFα, IL12, or IL6.[31] However, IL1 family cytokines, IL1α, IL1β, IL18, and IL1ra were higher in $SHIP^{-/-}$ ileal homogenates relative to $SHIP^{+/+}$ and the IL1ra/IL1 ratio was 3.6-fold lower in $SHIP^{-/-}$ ileal homogenates (Figure 1*A*). To investigate the cellular source of IL1β and IL18 in ileal tissues, tissue cross-sections were co-stained with F4/80, a macrophage marker, and YVAD-FLICA, which stains active caspase-1. YVAD-$FLICA^{+}$ cells were found in the subepithelial region of villi in $SHIP^{-/-}$ mice and co-stained with F4/80 (Figure 1*B*). Purified ileal macrophages from $SHIP^{+/+}$ and $SHIP^{-/-}$ mice were stimulated with LPS (5 hours), to induce IL1β and IL18 transcription, ATP (1 hour), to induce inflammasome activation; or LPS (5 hours) + ATP (for the last hour) and IL1β and IL18 production were measured. $SHIP^{-/-}$ ileal macrophages secreted IL1β and IL18 in response to LPS alone, consistent with staining for active caspase-1, and $SHIP^{-/-}$ ileal macrophages secreted significantly more IL1β and IL18 in response to LPS+ATP (Figure 1*C*).

In Vivo Differentiated $SHIP^{-/-}$ Macrophages Produce High Levels of Interleukin 1β and Interleukin 18

We next established an in vitro culture model to investigate the mechanism(s) for increased IL1β and IL18 secretion by $SHIP^{-/-}$ macrophages. BM progenitors were differentiated into macrophages in the presence of macrophage growth factors, macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, or IL3. Macrophages were stimulated with LPS, ATP, or LPS+ATP, and IL1β was measured in culture supernatants. Macrophage colony stimulating factor–derived or IL3-derived $SHIP^{-/-}$ BMMs did not produce more IL1β than $SHIP^{+/+}$ BMMs in response to stimulation. Granulocyte-macrophage colony-stimulating factor–derived $SHIP^{-/-}$

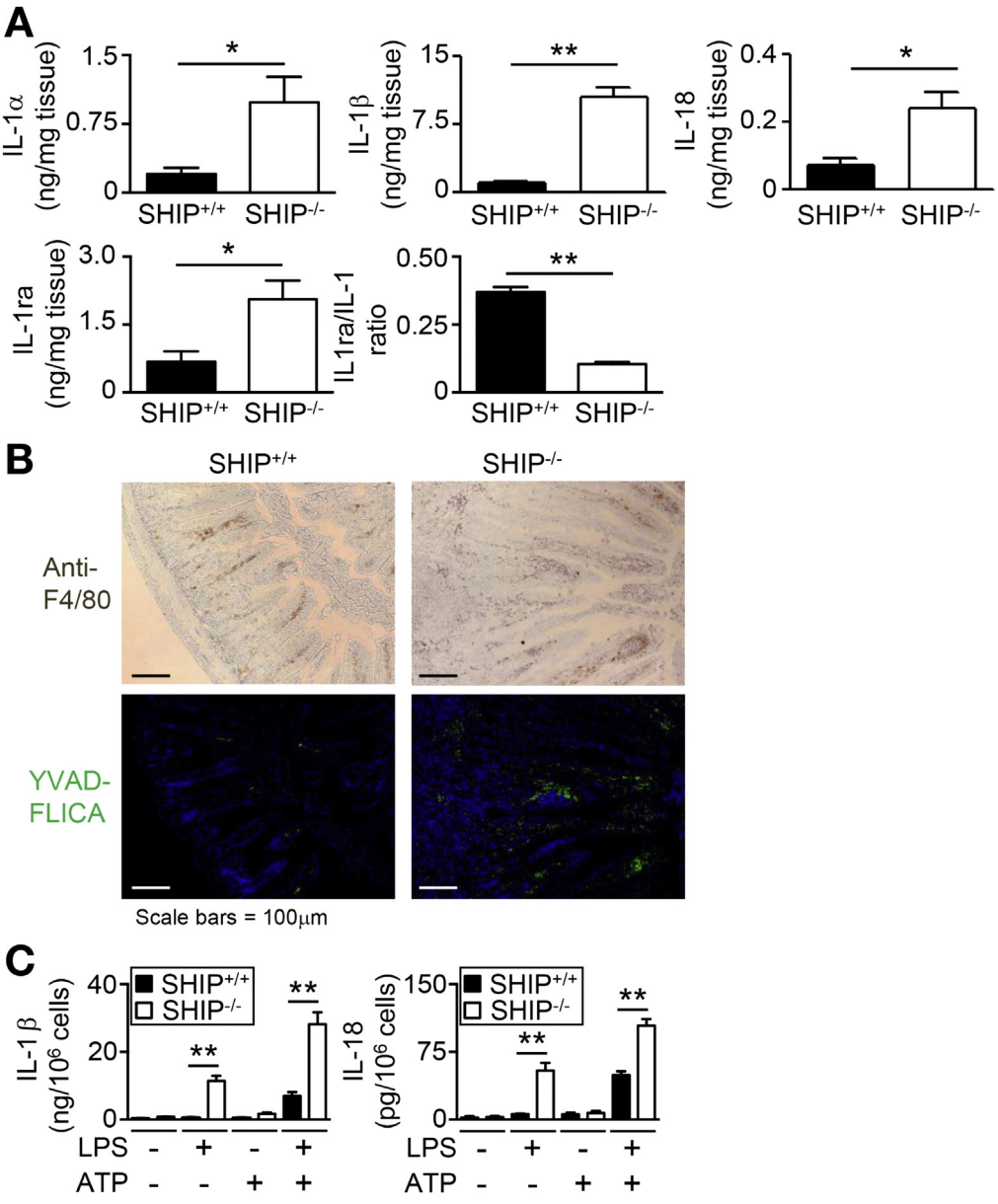

Figure 1. SHIP$^{-/-}$ ileal macrophages produce more IL1β and IL18 than SHIP$^{+/+}$ ileal macrophages. (*A*) Full-thickness ileal tissue homogenates from 8-week-old SHIP$^{+/+}$ and SHIP$^{-/-}$ mice were assayed for IL1 family cytokines by ELISA and the IL1ra/IL1 ratio was calculated. (*B*) Ileal sections were co-stained with anti-F4/80 for macrophages and with YVAD-FLICA for active caspase-1. Data are representative of sections from 6 mice/group. (*C*) Ileal mononuclear phagocytes were isolated and stimulated with LPS, ATP, or LPS+ATP. Supernatants were assayed for IL1β or IL18. Data are mean ± SD for n = 4 mice/group in (*A*) and n = 6 mice/group in (*C*). *$P < .02$; **$P < .01$.

BMMs produced more IL1β than those from SHIP$^{+/+}$ mice, but the effect was modest compared with the differences observed for ileal macrophages (Figure 2*A*). Given that SHIP$^{-/-}$ macrophages can be influenced by the complex inflammatory environment in the SHIP$^{-/-}$ ileum, Granulocyte-macrophage colony-stimulating factor–derived macrophages from SHIP$^{+/+}$ and SHIP$^{-/-}$ mice were treated for 3 days with interferon (IFN) γ, to induce a classically activated phenotype, IL4 (elevated in SHIP$^{-/-}$ ilea),[31] to induce an alternatively activated phenotype, or IFNγ+IL4. IFNγ and (IFNγ+IL4)-treated, granulocyte macrophage colony-stimulating factor–derived SHIP$^{-/-}$ BMMs produced more IL1β than SHIP$^{+/+}$ BMMs (Figure 2*B*), but did not replicate the dramatic differences in IL1β secretion seen in ileal macrophages. Reasoning that differences observed may be due to in vivo differentiation, SHIP$^{+/+}$ and SHIP$^{-/-}$ peritoneal macrophages were assessed. IL1β and IL18 secretion were significantly higher in SHIP$^{-/-}$ peritoneal macrophages compared to SHIP$^{+/+}$ peritoneal macrophages (Figure 2*C*). Peritoneal macrophages were also stimulated with LPS+ATP in the presence of glybenclamide to inhibit ATP-induced potassium channel efflux required for inflammasome activation, or YVAD, to inhibit caspase-1. Glybenclamide and YVAD blocked (LPS+ATP)-induced IL1β and IL18 secretion by SHIP$^{-/-}$ macrophages as effectively as in SHIP$^{+/+}$ macrophages (Supplementary Figure 1).

Class I PI3Kp110α Activity Increases Il1b Transcription

SHIP is a critical negative regulator of class I PI3Ks. To define the mechanism by which SHIP deficiency increases (LPS+ATP)-induced IL1β and IL18 secretion, SHIP$^{-/-}$ peritoneal macrophages were stimulated with LPS+ATP in

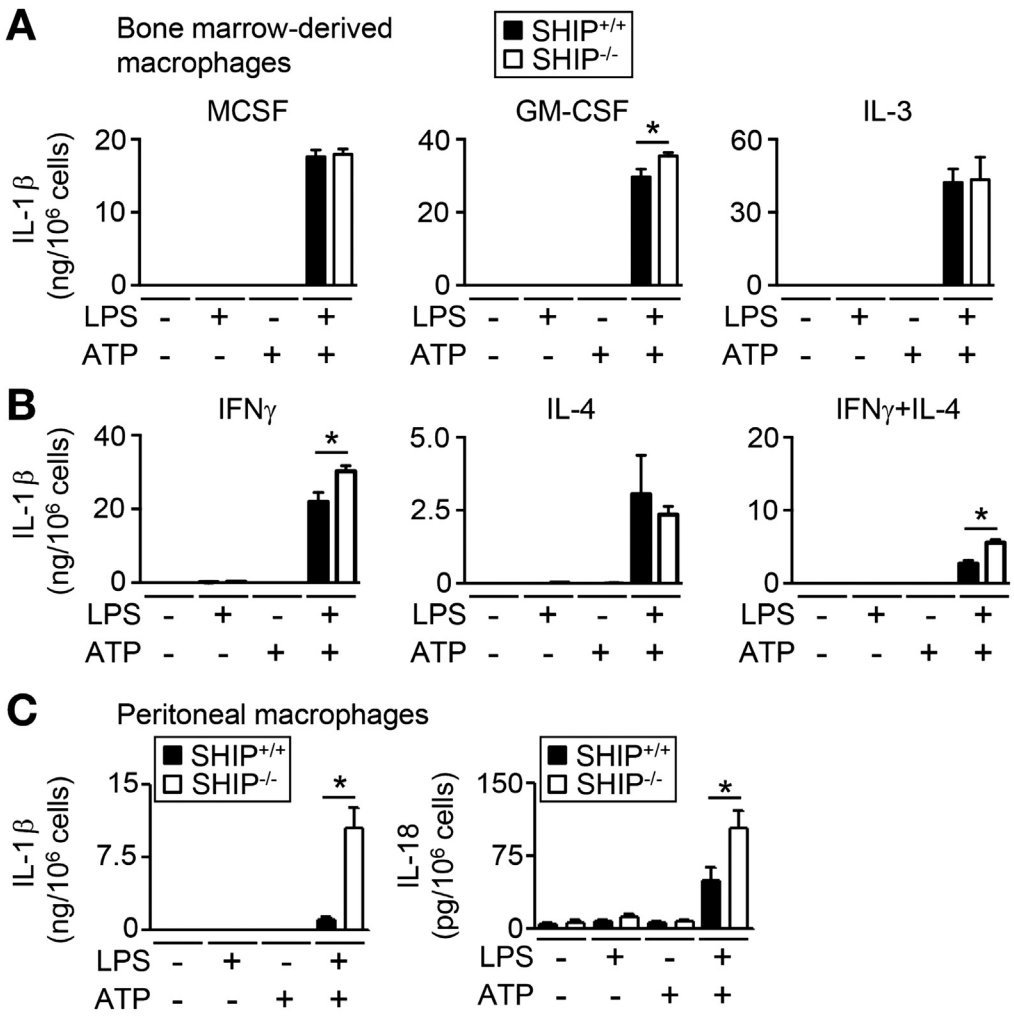

Figure 2. In vivo differentiated SHIP$^{-/-}$ macrophages produce high levels of IL1β and IL18. SHIP$^{+/+}$ and SHIP$^{-/-}$ BMMs were stimulated with LPS, ATP, or LPS+ATP and supernatants were assayed for IL1β. (*A*) BMMs differentiated for 10 days with 10 ng/mL macrophage colony stimulating factor, granulocyte macrophage colony-stimulating factor (GM-CSF), or IL3. (*B*) GM-CSF–derived BMMs treated for 3 days with 10 ng/mL IFNγ, IL4, or IFNγ+IL4. (*C*) Peritoneal macrophages from 8-week-old SHIP$^{+/+}$ and SHIP$^{-/-}$ mice. Data are mean ± SD for n = 4 in (*A*) and (*B*) and n = 6 in (*C*). *$P < .05$; **$P < .01$.

the presence of pan-PI3K inhibitors, LY294002 or Wm, or controls, LY303511 (an inactive structural analogue) or dimethyl sulfoxide (vehicle control). LY294002 and Wm blocked IL1β production when added to cultures before stimulation with LPS, but not when added to culture before addition of ATP, suggesting that class I PI3K is required for LPS-induced *Il1b* transcription, but not inflammasome activation (Figure 3*A*). Isoform-specific class I PI3Kp110α inhibitors, PIK-90 and PI-103, also reduced (LPS+ATP)-induced IL1β secretion, whereas inhibitors for other isoforms had no effect (Figure 3*B*). Indeed, SHIP$^{-/-}$ peritoneal macrophages and ileal tissues had higher *Il1b* gene expression compared with SHIP$^{+/+}$ littermates (Figure 3*C*). LY294002, Wm, and PI3Kp110α inhibitors reduced *Il1b* transcription in peritoneal macrophages from both SHIP$^{+/+}$ and SHIP$^{-/-}$ mice (Figure 3*D*).

Macrophage Depletion Reduces Intestinal Inflammation in SHIP$^{-/-}$ Mice

Ileal macrophages isolated from 8-week-old SHIP$^{-/-}$ mice are distinct from those isolated from SHIP$^{+/+}$ mice. There were 7.1-fold more CD11b^{hi} cells among viable CD45^{+}MHCII^{+}F4/80^{+} lamina propria cells. Among the quadruple-positive cells, there was an increase in chemokine receptor–positive cells, a 1.87-fold increase in CX3CR1^{+} cells, and a 17.8-fold increase in CCR5^{+} cells (Supplementary Figure 2).

To understand the contribution of macrophages to intestinal inflammation in SHIP$^{-/-}$ mice, we treated SHIP$^{-/-}$ mice with clod-lip to deplete macrophages for 2 weeks during disease development. Clod-lip–treated SHIP$^{-/-}$ mice had reduced gross and histologic pathology in the ileum (Figure 4*A*). Clod-lip depleted 55% ± 5% of macrophages in the ilea of SHIP$^{-/-}$ mice and eliminated staining for active capase-1 (Figure 4*B*). Macrophage depletion reduced histologic evidence of inflammation including crypt-villus hyperplasia and the number of immune cells in ileal sections (Figure 4*C*), and reduced IL1β and IL6 levels in ileal tissue homogenates (Figure 4*D*).

Anakinra Treatment Reduces Intestinal Inflammation in SHIP$^{-/-}$ Mice

Anakinra is an IL1 receptor antagonist that is used to treat autoinflammatory diseases. Like macrophage depletion, prophylactic treatment with anakinra effectively blocked the onset of disease (Supplementary Figure 3).

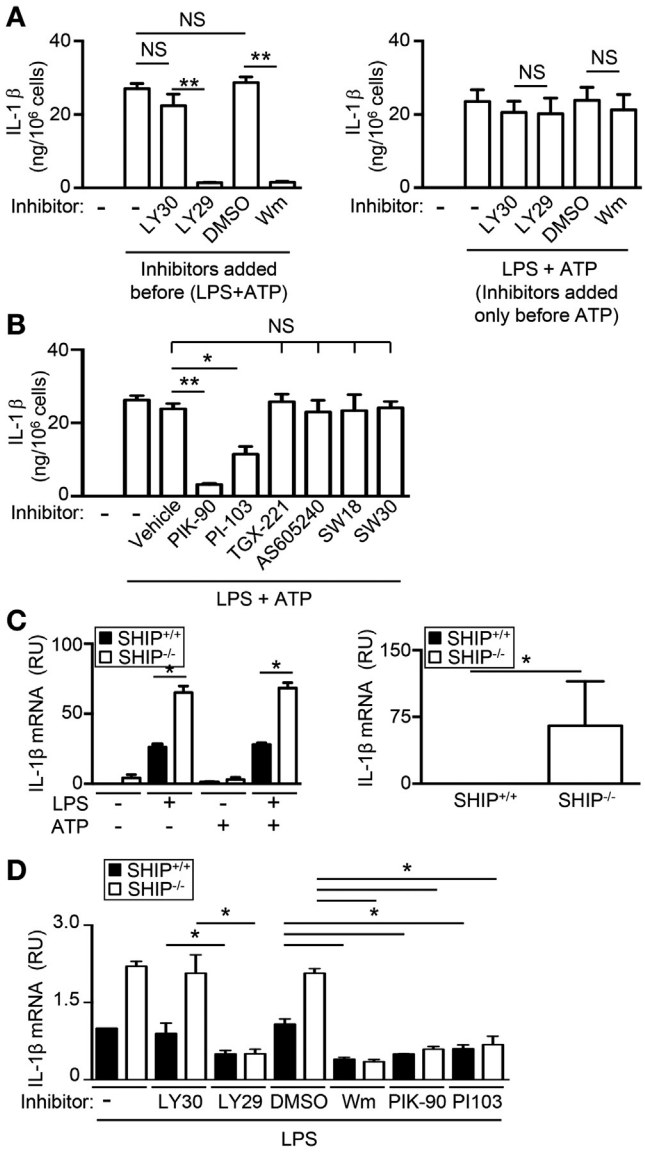

Figure 3. SHIP deficiency increases transcription of IL1β via class I PI3Kp110α activity. (*A*) SHIP$^{-/-}$ peritoneal macrophages were stimulated with LPS for 4 hours, followed by addition of ATP for 1 hour ± pan-PI3K inhibitor, LY294002 (LY29), its inactive analogue LY305311, pan-PI3K inhibitor, Wm, or dimethyl sulfoxide (DMSO), as a vehicle control. PI3K inhibitors or controls were added to cultures 30 minutes before LPS (*left*) or ATP (*right*) and cell supernatants were assayed for IL1β by ELISA. (*B*) SHIP$^{-/-}$ peritoneal macrophages were stimulated with LPS+ATP ±isoform-specific inhibitors for class I PI3Ks or DMSO (vehicle). IL1β was assayed in cell supernatants by ELISA. (C) SHIP$^{+/+}$ and SHIP$^{-/-}$ peritoneal macrophages were stimulated with LPS, ATP, or LPS+ATP. RNA was isolated from cells and *Il1b* transcription was assayed by quantitative polymerase chain reaction (*left*). RNA was isolated from ileal tissues of 8-week-old SHIP$^{+/+}$ and SHIP$^{-/-}$ mice and *Il1b* transcription was assayed (*right*). (*D*) SHIP$^{+/+}$ (*left*) and SHIP$^{-/-}$ (*right*) peritoneal macrophages were stimulated with LPS+ATP ± pan-PI3K inhibitors, LY29 or Wm, or PI3Kp110α isoform-specific inhibitors, PIK-90 or PI-103, or controls, LY303511 or DMSO. RNA was isolated from cells and *Il1b* transcription was assayed. Data are means ± SD for n = 6 in (*A*) and n = 4 in (*B*–*D*). *$P < .01$; *$P < .0001$.

To determine whether anakinra could be used to treat mice therapeutically, SHIP$^{-/-}$ mice were treated with anakinra or phosphate-buffered saline (injection control) from 6–8 weeks of age, beginning treatment after the establishment of ileal inflammation. SHIP$^{-/-}$ mice treated with anakinra had reduced gross and histologic pathology (Figure 5*A*). Anakinra reversed macrophage accumulation in the ileum and reduced *Il1b* gene expression in ileal tissues (Figure 5*B*). Anakinra did not reduce crypt-villus hyperplasia but reversed inflammatory markers, including the number of immune cells in ileal sections (Figure 5*C*), as well as levels of IL1β and IL6 in ileal tissue homogenates (Figure 5*D*). Anakinra also reduced fibrotic complications of disease in SHIP$^{-/-}$ mice reversing muscle thickening[31] and blocking collagen accumulation, measured by Sircol assay and Masson's trichrome staining (Figure 5*E*).

SHIP Activity Is Lower in Subjects With Crohn's Disease

To determine whether SHIP may play a role in intestinal inflammation in people with CD, we assayed SHIP in subjects with ileal CD and control subjects, who did not have IBD. Ileal and colonic biopsies and peripheral blood were collected from treatment-naïve subjects who were undergoing colonoscopy. SHIP protein levels and activity were significantly lower in ileal biopsies from subjects with CD compared with control subjects (Figures 6*A* and *B*). Supplementary Figure 4 demonstrates the efficiency of SHIP immunoprecipitation and 10-fold increased sensitivity of assaying SHIP activity compared with measuring protein levels. SHIP is hematopoietic-specific, so it is interesting to note that SHIP activity is lower in ileal tissues from subjects with CD despite a 2.2-fold increase in immune cells in CD subjects (Figure 6*C*). SHIP activity was also lower in PBMCs from subjects with CD compared with control subjects and SHIP activity in PBMCs inversely correlated with (LPS+ATP)-induced IL1β production (Figure 6*D*).

Discussion

Here we demonstrated that chronic ileitis in SHIP$^{-/-}$ mice is associated with elevated levels of macrophage-derived IL1β. Increased IL1β production occurs in in vivo differentiated SHIP$^{-/-}$ macrophages, which have increased class IA PI3Kp110α-driven *Il1b* transcription. Ileitis was prevented by macrophage depletion or reduced by treatment with the IL1 receptor antagonist, anakinra. Translating our findings to humans, SHIP protein levels and activity are lower in the inflamed ileum of treatment-naïve subjects with CD, and SHIP activity is reduced in their PBMCs and inversely correlates with the ability of their PBMCs to produce IL1β.

SHIP plays pleotropic roles in macrophage activation by limiting PI3K activity downstream of receptor stimulation. The PI3K pathway is activated downstream of TLRs and modulates downstream cytokine production.[37,38] PI3K is generally believed to negatively regulate proinflammatory cytokine production, but specific isoforms have positive or

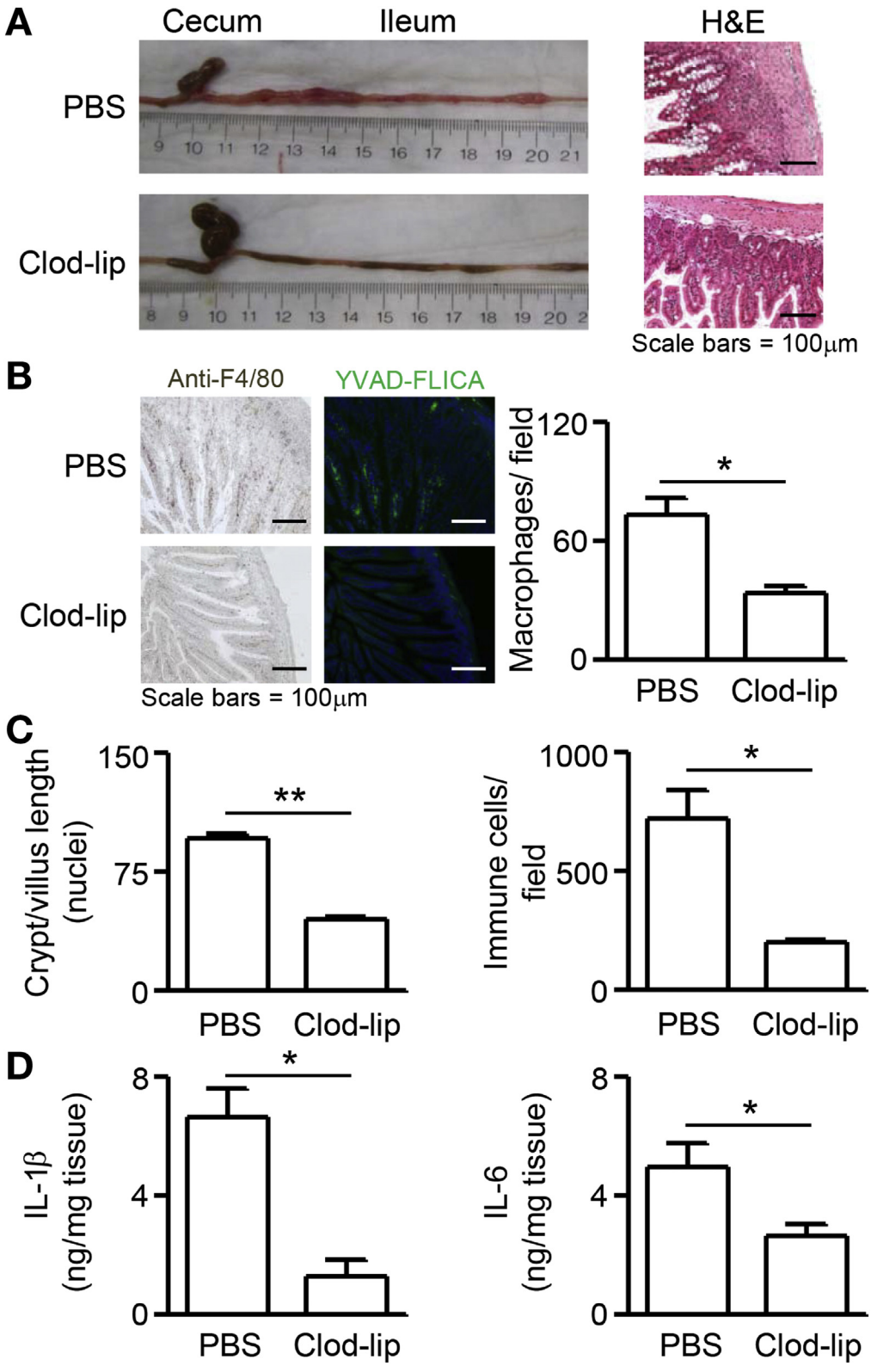

Figure 4. Macrophage depletion reduces development of intestinal inflammation in SHIP$^{-/-}$ mice. SHIP$^{-/-}$ mice were injected intraperitoneally with clod-lip or phosphate-buffered saline (injection control) from 4−6 weeks of age. (*A*) Gross pathology of distal ilea (*left*) and H&E-stained ileal cross-sections (*right*) of 6-week-old SHIP$^{-/-}$ mice. (*B*) Ileal cross-sections co-stained with anti-F4/80 (macrophages) and YVAD-FLICA (active caspase-1) (*left*) and quantitation of F4/80^{+} macrophages in ileal cross-sections. (*C*) Crypt-villus length (*left*) and quantification of immune cell infiltration (*right*). (*D*) IL1β (*left*) and IL6 (*right*) in full-thickness ileal tissue homogenates. Photographs in (*A*) and (*B*) are representative images from 6 mice/group. In (*B*) and (*C*), counting was performed on 6 mice/group counting 6 fields in 6 sections separated by $\geq$50 μm and by 2 individuals blinded to experimental condition. Data are mean $\pm$ SD for n = 6. *$P < .03$; **$P < .0001$.

negative regulatory activity. PI3Kp110α positively regulates IL12p40 and IL6 production by murine and human macrophages[28,39] and SHIP negatively regulates this TLR-induced cytokine production.[28,29] Herein, we demonstrate a critical role for SHIP in *Il1b* transcription where SHIP limits PI3Kp110α activity downstream of LPS/TLR4 signaling and contributes to intestinal inflammation. PI3K and its downstream targets, Akt and mTOR, are activated by TLR4

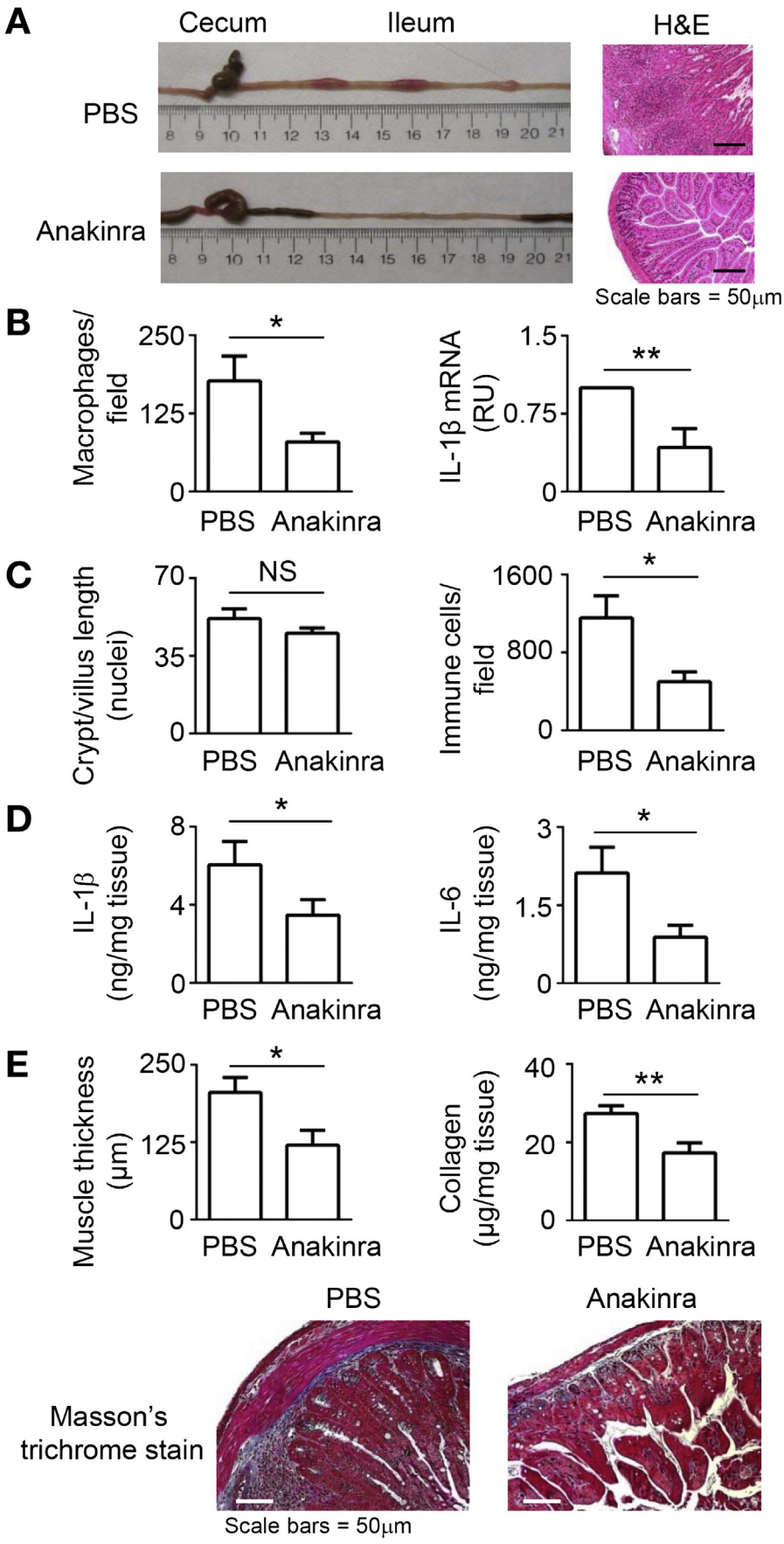

Figure 5. Anakinra treatment reduces intestinal inflammation in SHIP$^{-/-}$ mice. SHIP$^{-/-}$ mice were injected intraperitoneally with the IL1 receptor antagonist, anakinra, or phosphate-buffered saline, as an injection control, from 6–8 weeks of age, and assessed at 8 weeks of age. (*A*) Gross pathology of distal ilea (*left*) and H&E-stained ileal cross-sections (*right*). (*B*) Quantitation of F4/80^{+} macrophages in ileal cross-sections (*left*) and IL1β messenger RNA in full-thickness ileal tissue homogenates (*right*). (*C*) Crypt-villus length (*left*) and immune cell infiltration (*right*) quantitated by microscopy. (*D*) IL1β and IL6 in full-thickness ileal tissue homogenates. (*E*) Muscle thickness in ilea cross-sections (*left*), soluble collagen in ileal tissues measured by Sircol (*right*), and Masson's trichrome stained ileal cross-sections. Photographs in (*A*) and (*E*) are representative images from 9 mice/group. In (*B*), (*C*), and (*E*), counting was performed on 9 mice/group counting 6 fields in 6 sections separated by $\geq$50 μm and by 2 individuals blinded to experimental condition. Data are mean $\pm$ SD for n = 9. *$P < .05$; **$P < .0001$.

signaling and both contribute to increased *Il1b* transcription in SHIP$^{-/-}$ macrophages (data not shown). Additional evidence in the literature implicates the PI3K/SHIP axis in IL1β production and intestinal inflammation. The microRNA, miR-155, targets SHIP protein.[40] Triptolide amelioration of inflammation post-ileocolonic anastomosis in IL10$^{-/-}$ mice acts via decreasing miR-155 levels and correlates with increased SHIP protein levels, which is presumed to be the mechanism for attenuation of proinflammatory cytokine production.[41] miR-155 is also up-regulated during dextran sulfate sodium (DSS)—induced colitis[42] and spontaneous colitis in IL10$^{-/-}$ mice,[43] both of which have been associated with increased IL1β production. Finally, targeting mTOR, downstream of PI3K, effectively reduces inflammation during DSS—induced colitis in mice and has been used effectively to treat refractory CD.[44]

Our data demonstrate that SHIP deficiency alone is not sufficient to induce high IL1β production by macrophages. This is consistent with a report that showed that LysM-cre×SHIP$^{fl/fl}$ mice (SHIP deficient in myeloid cells including macrophages) did not develop the ileal inflammation.[45] SHIP$^{-/-}$ ileal macrophages are distinct from those isolated from SHIP$^{+/+}$ mice because more SHIP$^{-/-}$ macrophages express high levels of chemokine receptors CX3CR1 and CCR5. This suggests that high IL1β production by in vivo differentiated SHIP$^{-/-}$ macrophages may be due to recent infiltration and differentiation of blood monocytes and/or may require cell-extrinsic factors present in the complex inflammatory environment in germ-line SHIP$^{-/-}$ mice. These 2 possibilities can both contribute to the unique macrophage activation state in the SHIP$^{-/-}$ mouse ileum. Although the cell type that initiates the inflammatory response remains unknown, Kerr et al[32] reported that BM-derived hematopoietic cells drive ileitis in germ-line SHIP$^{-/-}$ mice because disease can be transferred to SHIP$^{+/+}$ mice by BM transplantation and can be cured in SHIP$^{-/-}$ mice by BM transplantation.[32]

SHIP activity inversely correlated with IL1β production in 3 model systems: in ex vivo isolated macrophages from mice, in ileal tissues from mice, and in PBMCs from human subjects.

In humans, the IL1ra/IL1 ratio is reduced in patients with CD compared with healthy control subjects and is proportional to disease activity.[10] The IL1ra/IL1 ratio was 3.6-fold lower in SHIP$^{-/-}$ mice compared with SHIP$^{+/+}$ mice, which is comparable with changes seen in patients with moderate CD.[10] However, the role of IL1β production in murine intestinal inflammation is considered controversial. In a model of acute intestinal inflammation in mice, DSS-induced colitis, concurrent studies reported that loss of

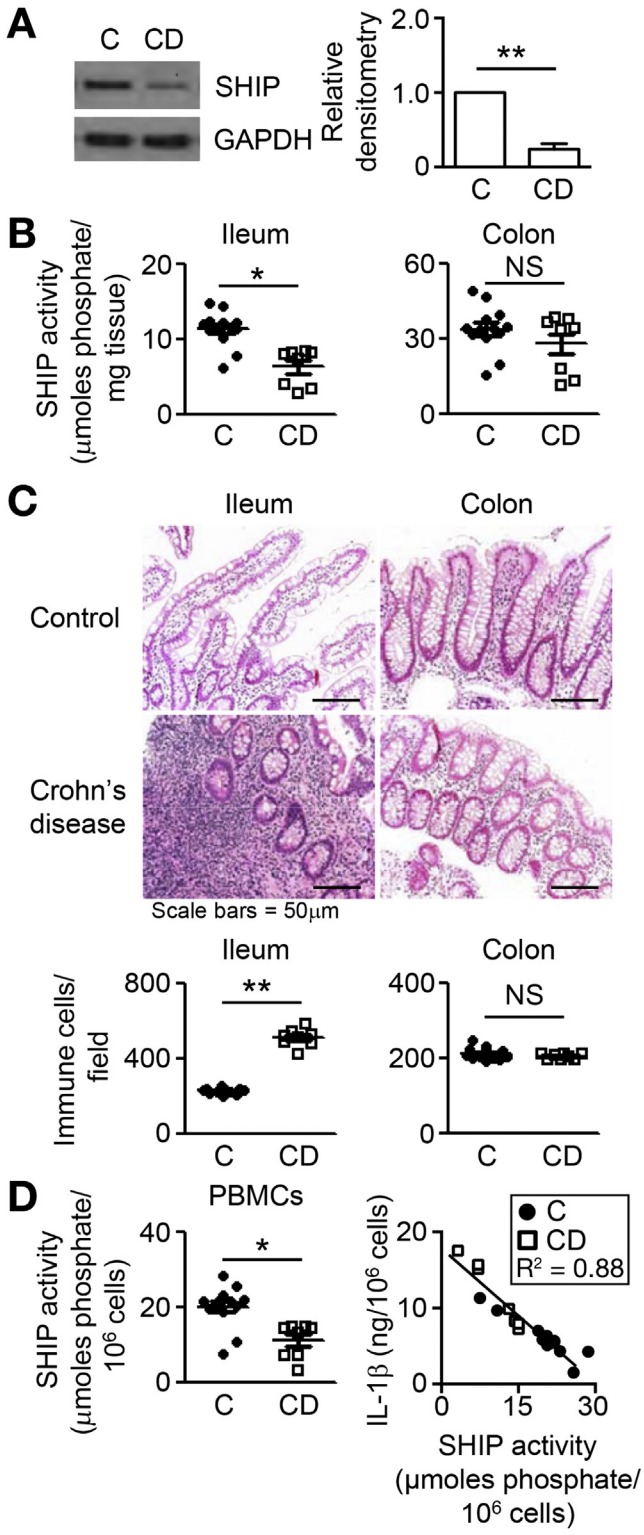

Figure 6. SHIP activity is lower in ileal biopsies and PBMCs from subjects with CD compared with control subjects and is inversely proportional to IL1β production by PBMCs. Ileal and colonic biopsies and PBMCs were collected from treatment-naïve subjects undergoing colonoscopy as part of their diagnosis; n = 8 subjects diagnosed with ileal CD and n = 11 subjects, who did not have IBD. SHIP protein expression (*A*) in ileal biopsies and SHIP activity (*B*) in ileal and colonic biopsies from control (C) subjects and subjects with CD (CD). (*C*) H&E-stained ileal and colonic biopsies and quantitation of immune cells. (*D*) SHIP activity in PBMCs from control subjects (C) and subjects with CD (CD; *left*). (LPS+ATP)-induced IL1β production by PBMCs vs SHIP activity in control subjects (*black circles*) and subjects with CD (*open squares; right*).

the NLRP3 inflammasome reduced[46] or exacerbated[47] intestinal inflammation. Consistent with the former report, caspase-1 inhibition[46] or caspase-1 deficiency,[48] protected mice from DSS-induced colitis. Consistent with the latter report, NLRP6[49] or IL1β[50] deficiency exacerbated DSS-induced colitis in mice. These discrepancies may be attributed to dysbiosis during intestinal inflammation[49] and/or a role for macrophage-derived IL1β in tissue restitution post–DSS-induced epithelial cell injury.[50] In contrast, the role of IL1β during chronic intestinal inflammation is not controversial. Caspase-1 deficiency protects mice during chronic DSS-induced inflammation;[48] TLR5$^{-/-}$ mice treated with a neutralizing IL10 receptor antibody develop spontaneous IL1β/IL1-receptor–dependent colitis[51] and inflammasome activation and IL1β production contribute to spontaneous IL1-receptor–dependent and caspase-1–dependent intestinal inflammation in IL10$^{-/-}$ mice.[52] Our data also suggest that IL1β contributes to chronic intestinal inflammation in mice because reducing IL1β by macrophage depletion or anakinra treatment ameliorated spontaneous intestinal inflammation in SHIP$^{-/-}$ mice. Anakinra blocks IL1 signaling through the IL1 receptor, which can drive *Il1b* transcription, but does not interfere with IL1β production directly. Prophylactic or therapeutic anakinra treatment in SHIP$^{-/-}$ mice led to a dramatic decrease in IL1β messenger RNA, which is consistent with it blocking the autoamplification of IL1β.

Finally, SHIP protein levels and activity were reduced in ileal biopsies and PBMCs from treatment-naïve human subjects with ileal CD compared with control subjects. This is particularly compelling, given that SHIP expression is restricted to hematopoietic cells and there is a dramatic influx of hematopoietic cells into intestinal tissue in patients with IBD. Arijs et al[53] reported that SHIP messenger RNA levels are increased in colonic biopsies from IBD subjects with ulcerative colitis or Crohn's colitis, but are unaffected in ileal biopsies from subjects with ileal CD.[53] Our data advance these observations, demonstrating that SHIP protein levels and activity are reduced in subjects with ileal inflammation. SHIP is up-regulated in response to MyD88-dependent TLR signaling,[28,29] which provides a mechanism for increased SHIP messenger RNA levels in the colon where commensal micro-organisms are abundant. SHIP protein levels and activity are also reduced post-transcriptionally after activation, which trigger SHIP's degradation by the proteasome.[30,54] This suggests a model in which SHIP is activated to reduce PI3K signaling during intestinal inflammation, but is ultimately degraded in the ileum exacerbating inflammation.

In conclusion, it has been suggested that CD may encompass multiple subtypes of disease. In addition, CD has been included among polygenic disease associated with autoinflammation due to overlapping features, which include its periodicity, strong association with environmental triggers, and failure to respond to therapy directed against TNFα in some patients.[55] Our mouse studies suggest that SHIP suppresses macrophage-derived IL1β production and contributes to ileal inflammation in the SHIP$^{-/-}$ mouse, which can be effectively treated with the IL1 receptor antagonist, anakinra. Our human studies further suggest that SHIP activity is reduced in subjects with ileal CD. Low SHIP activity inversely correlates with elevated IL1β production by macrophages and might be a useful biomarker to identify a subpopulation of patients with CD who are amenable to treatment targeting IL1.

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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.09.049.

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The authors disclose no conflicts.

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